Janssen Vaccines & Prevention B.V.*

Clinical Protocol

Protocol Title

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

HORIZON 1

Protocol VAC31518COV2004; Phase 2

AMENDMENT 7

VAC31518 (JNJ-78436735)

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

United States (US) sites of this study will be conducted under US Food & Drug Administration Investigational New Drug (IND) regulations (21 CFR Part 312).

IND: 22657

EudraCT NUMBER: 2020-005330-14

Status: Approved
Date: 16 September 2022
EDMS number: EDMS-RIM-194495, 10.0

GCP Compliance: This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Amendment 7	**This document**
Amendment 6	05 May 2022
Amendment 5	18 March 2022
Amendment 4	13 December2021
Amendment 3	02 July 2021
Amendment 2	19 March 2021
Amendment 1	28 February 2021
Original Protocol	07 December 2020

Amendment 7 (This Document)

Overall Rationale for the Amendment:

The purpose of the Amendment is to cease further enrollment in this study. After approximately 10 months, recruitment continues to be challenging despite of the various Protocol Amendments intended to increase enrollment. In addition, there is a lack of participant interest in this study, partly because of the widespread use of other COVID-19 vaccines that are recommended for use in pregnant women. The participants and their infants enrolled up to Amendment 7 will continue to be followed in this study as per protocol.

Because of the aforementioned enrollment challenges, the limited number of participants that are currently included, and the availability of pregnancy registry data, some sampling time points are being removed to reduce the burden on the participants. Changes made to the clinical protocol of study VAC31518COV2004 as part of Amendment 7 are listed below, including the rationale for each change and a list of all applicable sections. Changes made as apart of Protocol Amendments 1 to 6 are listed in Appendix 10.14.

Section number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.3.1 Adult Participants: Group 4: (Including Sentinel and Safety Cohorts) 1.3.2 Adult Participants: Groups 1-3: Previously Vaccinated Participants: Dose 1 Schedule 4.1 Overall Design 6.3 Preparation/Handling/Storage/Accountability 9.2 Sample Size Determination 9.2.1 Immunogenicity	Clarified that further enrollment in this study is being ceased as of Amendment 7.	Due to enrollment challenges detailed above.
1.1 Synopsis 1.3.1 Adult Participants: Group 4: (Including Sentinel and Safety Cohorts) 1.3.2 Adult Participants: Groups 1-3: Previously Vaccinated Participants: Dose 1 Schedule 9.2 Sample Size Determination 9.2.1 Immunogenicity	Blood samples from adult participants for immunogenicity were removed from the end of study and early exit visits and the name of the visit was updated in the table accordingly. Updated wording for follow-up safety assessments for adults at the end of the study visit (no.12)	To reduce the burden on the participants.
1.1 Synopsis 1.3.3 Neonate/Infants Schedule 3 OBJECTIVES AND ENDPOINTS	Blood samples collection from neonates at 12 months for immunogenicity were removed	To reduce the burden on the participants.

Section number and Name	Description of Change	Brief Rationale
4.1.1 Study-Specific Ethical Design Considerations 8 STUDY ASSESSMENTS AND PROCEDURES	and the name of the visit was updated in the table accordingly. Updated wording for postnatal (PN 12 months) follow-up safety visit (no. 14).	
1.1 Synopsis 1.3.1 Adult Participants: Group 4: (Including Sentinel and Safety Cohorts) 1.3.2 Adult Participants: Groups 1-3: Previously Vaccinated Participants: Dose 1 Schedule 3 OBJECTIVES AND ENDPOINTS 4.1 Overall Design 8 STUDY ASSESSMENTS AND PROCEDURES 8.1.1 Immunogenicity Assessments 9.4.3 Exploratory Endpoint(s)	PBMC sampling was removed from the study.	To reduce the burden on the participants As only 1 participant was currently enrolled in the peripheral blood mononuclear cells (PBMC) subset, this will result in insufficient data to draw any conclusions.
1.1 Synopsis 9.4 Statistical Analyses 9.4.1 General Considerations 9.5 Planned Analyses 9.5.2 Final Analysis	The interim analyses that were planned when 120 and 240 participants reached the 28 days post-vaccination visit were removed from the study.	Due to enrollment challenges as described above the anticipated target required for interim analyses will not be reached.
1.1 Synopsis 9.4 Statistical Analyses	Updated wording regarding endpoints in alignment with Statistical Analysis Plan (SAP) details.	Due to enrollment stop and limited sample size.
General	Textual adjustments and edits.	Clarification and correction of minor inconsistencies.

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES AND FIGURES

TABLES

FIGURES

1. PROTOCOL SUMMARY

1.1. Synopsis

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

Ad26.COV2.S (also known as Ad26COVS1) is a monovalent vaccine composed of a recombinant, replication-incompetent adenovirus type 26 (Ad26) vector, constructed to encode the spike (S) protein derived from a severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) clinical isolate (Wuhan, 2019, whole genome sequence NC 045512), stabilized in its prefusion conformation.

OBJECTIVES AND ENDPOINTS

The primary and secondary objectives and endpoints of the study are:

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, ie, 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 booster vaccination. • SAEs and AESI throughout the study (from vaccination until end of the study, ie, at least 12 months after delivery). • MAAEs until 6 months after vaccination.

Objectives	Endpoints
	• 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 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 (ie, 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 (ie, 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 (ie, in cord blood).	• Serological response to vaccination as measured by VNA titers at birth (ie, in cord blood).
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 approximately 12 months of age. • MAAEs in neonates and infants from birth until 6 months of age.

Objectives	Endpoints
	• 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).

For exploratory endpoints, please refer to Section 3.

Due to cease of enrollment and limited sample size, some endpoints might not be summarized and will only be provided in listings. More details can be found in the SAP.

Hypothesis

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.

The study will provide descriptive information regarding the safety and immunogenicity of Ad26.COV2.S administered as a booster dose in participants who had previously received a coronavirus disease-2019 (COVID-19) vaccine and in those that were vaccine naïve at study entry and receive a booster dose during the study.

OVERALL 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.

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 in this study. Efforts were made to ensure good representation in terms of race and ethnicity.

As of Protocol Amendment 7 approval, participant enrollment in this study will be ceased. The enrolled participants and their infants will continue to be followed in this study in accordance with the protocol.

The first 22 vaccine naïve participants participated in the Sentinel and Safety Cohorts to assess safety and reactogenicity in the 1-dose regimen.

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) 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

The sample size of each of the groups will be flexible, and the target will be to recruit at least 40 participants in Groups 1-3 and at least 25 participants in Group 4. These participants will receive 1 dose of Ad26.COV2.S at 5×10^{10} vp. Participants will be 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.

Enrollment was staggered and started with recruitment of Sentinel participants (n=5) followed by a larger Safety Cohort (n=17 participants, excluding sentinels) who all received 1 dose of Ad26.COV2.S vaccine at 5×10^{10}vp.

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

Independent Data Monitoring Committee (IDMC) Review Outcome

Safety Profile Acceptable After IDMC Review

At the time of writing Amendment 5, 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 following review of the Sentinel and Safety Cohorts safety data (see Section 10.3.6 for details).

Upon recommendation of the IDMC, the remaining participants were enrolled to receive 1 dose of Ad26.COV2.S at 5×10^{10} vp.

Booster Vaccinations

Previously Vaccinated Participants

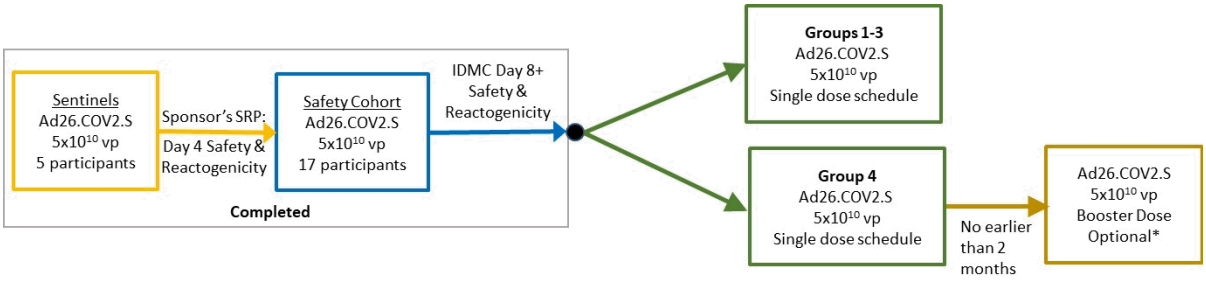
Previously vaccinated participants (Groups 1-3) will receive a single (booster) dose of Ad26.COV2.S at 5×10^{10} vp as part of the study. No further vaccinations will be received by these groups during the study.

Vaccine Naïve Participants

For participants that are vaccine naïve at study entry, booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp will be offered to ongoing, consenting participants (Group 4, including Sentinel and Safety Cohorts) who have completed the pregnancy during which they were enrolled in the study, are not pregnant again, and have not 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.

Decision Tree for Staggered Enrollment



Abbreviations: IDMC Independent Data Monitoring Committee; vp virus particles. SRP Study Responsible Physician

A target of 240 adult participants (including 22 participants in the Sentinel and Safety Cohorts) in the 2nd or 3rd trimester of pregnancy (Week 16 to Week 38 of gestation, inclusive) was to be enrolled. Enrollment was staggered and started with recruitment of Sentinel participants followed by Safety Cohort (excluding Sentinels).

At the time of writing Amendment 5, 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 following review of the Sentinel and Safety Cohorts safety data.

As of Protocol Amendment 7 approval, participant enrollment in this study will be ceased. The enrolled participants and their infants will continue to be followed in this study in accordance with the protocol.

The participants that were not enrolled in the Sentinel and Safety Cohorts will be enrolled and randomized to receive 1 dose of Ad26.COV2.S at 5×10^{10} vp.

Previously vaccinated participants (Group 1-3) should have received their last COVID-19 vaccination at least 4 months prior to receiving the study vaccine.

Participants will be 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.

* A booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp will be offered to ongoing, consenting participants who were vaccine naïve at study entry (Group 4, including Sentinel and Safety Cohorts) and who have completed the pregnancy during which they were enrolled in the study, are not pregnant again), and have not received another COVID-19 vaccine (eg, national immunization program). Please refer to Section 1.3.1.1 for further details.

Safety assessments include an observation period at the study site for at least 30 minutes (60 minutes for participants in the Sentinel and Safety Cohorts) 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), AESI, pregnancy-related AEs throughout pregnancy, and 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.

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

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]).

All participants and neonates/infants with COVID-19-like signs or symptoms and all participants and neonates/infants with a positive RT-PCR test from outside the study meeting the prespecified criteria for suspected COVID-19 should undertake prespecified COVID-19 procedures as outlined in the body of the protocol (see Section 8.1.2).

If neonates were born to mothers with confirmed COVID 19 (or if a close relative from the same household has a confirmed COVID 19 result), testing for SARS-CoV-2 should be performed for neonates/infants, regardless of the presence of symptoms.

The occurrence of asymptomatic SARS-CoV-2 infection will also be assessed, if feasible (see Section 8.1.4).

Enrolled participants will be counselled on SARS-CoV-2 infection prevention each time they interact with site staff, in line with local guidelines. All necessary precautions (per local regulation) should be taken to protect medical staff and other contacts of participants who confirmed to have COVID-19.

In the event of a confirmed SARS-CoV-2 infection, the participant will be asked to adhere to the appropriate measures and restrictions as defined by local regulations.

Study Population

A target of 240 adult participants, who are previously vaccinated or vaccine naïve at study entry, ≥18 to ≤45 years of age, inclusive, in the 2nd and/or 3rd trimester of pregnancy (ie, Week 16 to Week 38 of gestation, inclusive) was to be recruited for the study.

Efforts were made to ensure good representation in terms of race and ethnicity and to have at least approximately 25% participants in each trimester of the pregnancy (Weeks ≥16 to <28 or Weeks ≥28 to ≤38) per group.

As of Protocol Amendment 7 approval, participant enrollment in this study will be ceased

End of Study Definition

The end of study is considered as the last visit for the last participant in the study. For adult participants, the last visit is at approximately 12 months postpartum. For neonates/infants the last visit is at approximately 12 months of age.

NUMBER OF PARTICIPANTS

A target of 240 adult participants, who are previously vaccinated or vaccine naïve at study entry, in the 2nd or 3rd trimester of pregnancy, ≥18 to ≤45 years of age was planned to be enrolled in this study.

As of Protocol Amendment 7 approval, participant enrollment in this study will be ceased.

DOSAGE AND ADMINISTRATION

Participants will be vaccinated at the study site according to the schedule detailed in the overall design.

Ad26.COV2.S will be supplied at a concentration of 1×10^{11} vp/mL, in single-use vials, with an extractable volume of 0.5 mL. Formulation buffer will be supplied as diluent as 15 mM citrate, 5% (w/w) hydroxypropyl-β-cyclodextrin, 0.4% (w/w) ethanol, 0.03% (w/w) polysorbate 80, 75 mM NaCl, pH 6.2.

The vaccine will be extracted from the vial as follows: Ad26.COV2.S at 5×10^{10} vp dose level: 0.5 mL is withdrawn from 1 vial containing 0.75 mL 1×10^{11} vp/mL.

IMMUNOGENICITY EVALUATIONS

Blood Sampling:

From all adult participants (venous blood), neonates (cord blood at birth) and infants (venous or arterial blood) samples will be collected at selected timepoints for humoral immunogenicity assessments (Section 8.1.1), with an emphasis on neutralizing and binding antibody responses.

Biomarker analysis in PAXgene® tubes may be performed to explore potentially informative biomarkers related to vaccine immunogenicity.

For adult participants who receive a booster vaccination, blood samples will be collected pre-boost and 28 days post-boost for humoral immunogenicity assessments and vaccination-related biomarkers (Section 8.1.1).

From a subset of approximately 25 of adult participants per group, peripheral blood mononuclear cells (PBMC)s were to be collected for analysis of cellular immunogenicity (including T helper cell subsets [Th1/Th2] assessments). With only 1 participant in this subset at the time of writing Amendment 7, PBMC sampling will be removed from the study as of Protocol Amendment 7 approval.

A serologic test for past infection with SARS-CoV-2 will be performed per the timepoints provided in the SoA.

Colostrum and Breast Milk Sampling

Colostrum and breast milk will be collected, if feasible, from adult participants postpartum for the assessment of protective maternal humoral immunity, including SARS-CoV-2 binding immunoglobulins and IgA antibodies. Colostrum and/or breast milk will also be collected, if feasible, at the time of booster vaccination and 28 days post booster vaccination, in participants who were vaccine naïve at study entry and receive a booster after pregnancy completion.

Immunogenicity assessments may include, but are not limited to, the humoral and cellular immunogenicity assays (as available and feasible) summarized in the below table.

Summary of Humoral and Cellular Immunogenicity Assays

Summary of Humoral Immunogenicity Assays	
Assay	**Purpose**
Primary/Secondary endpoints	
SARS-CoV-2 binding antibodies (ELISA or equivalent assay)	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
SARS-CoV-2 binding immunoglobulins, including IgA antibodies (ELISA or equivalent assay)	Analysis of IgA and/or other Ig subtypes against SARS-CoV-2 in colostrum and/or breast milk
Adenovirus 26 neutralization (neutralization assay)	Analysis of neutralizing antibodies to Adenovirus 26
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 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, including innate and inflammatory responses

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, IL2, TNFα, IL-4, IL-5, IL13, 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 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 CoV2 severe acute respiratory syndrome coronavirus 2; Th T helper; Th1/Th2:T helper cell subset(s); TNFα tumor necrosis factor alpha; VNA virus neutralization assay.

BIOMARKER EVALUATIONS

For all adult participants and in cord blood (if feasible), biomarker analysis in PAXgene® tubes may be performed to explore potentially informative biomarkers related to vaccine immunogenicity.

For adult participants with a positive test result for SARS-CoV-2 infection, biomarker analysis in PAXgene® tubes may 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.

SAFETY EVALUATIONS

Day 1 Supplies:

Adult participants will be provided with a thermometer (to measure body temperature), ruler (to measure local injection site reactions), and a participant reactogenicity diary in the eCOA to record body temperature and solicited local (at injection site) and systemic signs and symptoms. Parent(s)/caregiver(s) will be provided with a separate thermometer for the neonate/infant to record body temperature for the neonate/infant in the eCOA in the event of confirmed or suspected COVID-19.

The site staff will be provided with ePRO and ObsRO completion guidelines that includes instructions on capturing responses the eCOA diaries and grading scales to assess severity of the signs and symptoms after vaccination (reactogenicity).

The study staff is responsible for providing appropriate training to the participant/parent(s)/caregiver(s) to prevent missing or incorrect data. The reactogenicity diary will be reviewed by the study personnel at the Day 8 visits as indicated in the Schedule of Activities (Section 1.3.1 and Section 1.3.2).

If the reactogenicity diary review is missed/unavailable at Day 8, the reactogenicity diary will be reviewed during the following visit.

Adult participants will also be provided with a kit to collect nasal swabs in case they experience COVID-19-like symptoms during the study.

Safety Assessments

After vaccination, participants will remain under observation at the study site, for at least 30 minutes (60 minutes for the Sentinel and Safety Cohorts), for the presence of any severe acute reactions and solicited events.

Vital signs, including body temperature (oral route preferred for adults, in accordance with the local standard of care for neonates/infants), pulse/heart rate, respiratory rate, blood pressure (adults only), and blood oxygen saturation will be assessed.

Physical examinations will be performed by the investigator or designated medically qualified healthcare professional. Any clinically relevant abnormalities or changes in severity observed during the review of body systems should be documented in the electronic case report form (eCRF).

General medical history will be documented in the eCRF, including previous surgery, hospitalizations, and medication history prior to conception/pregnancy.

Obstetric medical history will be documented in the eCRF, if available, including number of live births, stillbirths, abortions, c-sections, multiple pregnancies and complications if applicable. Any visits and results of any routine tests obtained during pregnancy to assess pregnancy and the fetus (eg, congenital anomalies, ultrasound, amniocentesis) should be documented. For prior pregnancies, dates of delivery or termination of pregnancy, history of multiple pregnancies, pregnancy complications, history of caesarean section (elective or emergency), pregnancy outcome(s) (live birth, still birth or abortion) and history of previous early-onset neonatal infection, if feasible.

Neonatal medical history will include birth/termination outcome (live birth, stillbirth, termination/miscarriage, neonatal death), demographics, physical examination, overall Apgar score, weight, length, head circumference, key findings of fetal monitoring during labor such as fetal presentation at delivery, gestational age, complications, fetal heart rate and uterine contractions during labor.

All AEs and special reporting situations, whether serious or non-serious, that are related to study procedures or that are related to non-investigational sponsor products will be reported for all participants (adult participants and neonates/infants) from the time a signed and dated informed consent form (ICF) is obtained until the end of the study/early withdrawal.

For adult participants, solicited AEs, collected through a reactogenicity diary as part of the eCOA, will be recorded from the time of vaccination until 7 days post-vaccination (or until resolution). All other unsolicited AEs and special reporting situations, whether serious or non-serious, will be reported from the time of vaccination until 28 days post-vaccination.

Subjective changes in (postpartum) breast milk production (reduction) will be collected in the eCOA diary.

All SAEs, AESI and AEs (including pregnancy-related AEs, throughout pregnancy) leading to discontinuation from the study/vaccination (regardless of the causal relationship) are to be reported for all adult participants from the moment of vaccination until completion of the participant's last study-related procedure. The investigator's assessment of ongoing AEs at the time of each participant's last visit should be documented and closed in the participant's medical record.

SAEs and AESIs in the neonates/infants will be followed up from birth until 12 months postpartum. MAAEs are to be reported for all adult participants from the moment of vaccination until 6 months after vaccination. MAAEs leading to study discontinuation (for both adults and neonates/infants), are to be reported during the entire study.

Plasma and serum derived from whole blood samples will be collected from all participants for retrospective analysis of hematological parameters, including pro- and anti-coagulation factors (see Section 8.2.3.1), in the event of a suspected AESI of thrombosis with thrombocytopenia syndrome (TTS) post-vaccination.

The platelet count will be performed by a local laboratory or substitute for local laboratory. In addition, the extent to which levels of these coagulation factors fluctuate pre-and post-vaccination with Ad26.COV2.S may be investigated for research purposes.

STATISTICAL METHODS

Sample Size Calculation

The number of adult participants that will be assessed for safety and reactogenicity of the Ad26.COV2.S vaccine, was chosen to provide a preliminary descriptive assessment of safety and immunogenicity in this population.

In addition, the study will provide descriptive information regarding the safety and immunogenicity of Ad26.COV2.S administered as a booster in eligible participants.

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. As of Protocol Amendment 7 approval, participant enrollment in this study will be ceased.

Descriptive analysis will be used to evaluate the following:

- Safety and reactogenicity
- Pregnancy outcomes
- Neonate/infant outcomes
- Immunogenicity of Ad26.COV2.S in adult participants and neonates/infants (including immunity conferred by placental transfer of IgG antibodies).

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.

Populations for Analysis Set

For purpose of analysis, the following populations are defined:

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. In addition, samples obtained from participants after SARS-CoV-2 infection occurring after baseline (if applicable) will be excluded from the analysis
Per protocol Immunogenicity- Non-vaccinated Neonates/Infants (PPI-NVN)	All 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 (for example missed vaccinations in the mothers), or neonates/infants with major protocol deviations that are expected to impact the immunogenicity outcomes

Population	Description
The list of major protocol deviations to be excluded 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.	

Primary/Secondary Endpoint(s)

Immunogenicity Endpoints

No formal statistical testing of immunogenicity data is planned. Descriptive statistics (geometric mean and confidence intervals, or median and interquartile range Q1-Q3, as appropriate) will be calculated by group and overall, for immunologic parameters for all available time points for adult participants receiving Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy.

Descriptive statistics will also be presented for neonates/infants of adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy by group and overall.

Several definitions of serological responses will be applied (fold increases in GMC [S-ELISA] or GMT [VNA]). Graphical representations of immunologic parameters will be prepared as applicable. Frequency tabulations will be calculated for discrete (qualitative) immunologic parameters, as applicable.

The impact of baseline characteristics on the humoral responses may be explored graphically or with descriptive statistics by group and overall.

For adult participants, all immunogenicity analyses will be performed on the PPI-A by group (previous vaccination status) and overall. Immunogenicity analyses may also be performed by stage of pregnancy at which the vaccine was administered, vaccination regimen (ie, 1-dose schedule or 1-dose schedule with a booster dose), SARS-CoV-2 baseline serostatus and by actual vaccine history within each group.

For neonates/infants, immunogenicity analyses will be performed on the PPI-NVN set by group and overall. Analyses may also be performed by the gestational age at the time of vaccine administration, and SARS-CoV-2 baseline serostatus of the adult participant.

Safety Endpoints

No formal statistical testing of safety data is planned. Safety data will be analyzed descriptively for adult participants (including pregnancy outcomes) and their neonates/infants up to approximately 12 months of age (including neonatal outcomes) for all enrolled participants.

For adult participants, all safety analyses will be performed on the Full Analysis Set-Adults (FAS-A).

For adult participants, safety analyses may also be performed by stage of pregnancy at which the vaccine was administered, vaccination regimen (ie, 1-dose schedule or 1-dose +booster), SARS-Cov-2 baseline serostatus and by actual vaccine history within each group.

For neonates/infants, safety analyses will be performed on the FAS-NVN.

For neonates/infants, analyses may also be performed by vaccination regimen (1-dose schedule or 1-dose +booster) received by the adult participant during the study.

Outcomes

Adverse maternal/fetal outcomes and adverse neonate/infant outcomes will be defined using the Case Definitions for Pregnancy Outcomes for each endpoint.

Planned Analyses

Primary Analysis

The primary analysis of safety and immunogenicity will be performed when all adult participants have completed the 42-day postpartum visit in all groups (or for participants who discontinued earlier).

The analysis will include safety (AEs, SAEs, AESIs, pregnancy-related AEs throughout pregnancy and pregnancy outcomes) and immunogenicity data (ELISA and VNA) 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 the Day 42 postpartum follow-up visit (inclusive). The results of this analysis may be used for regulatory submissions.

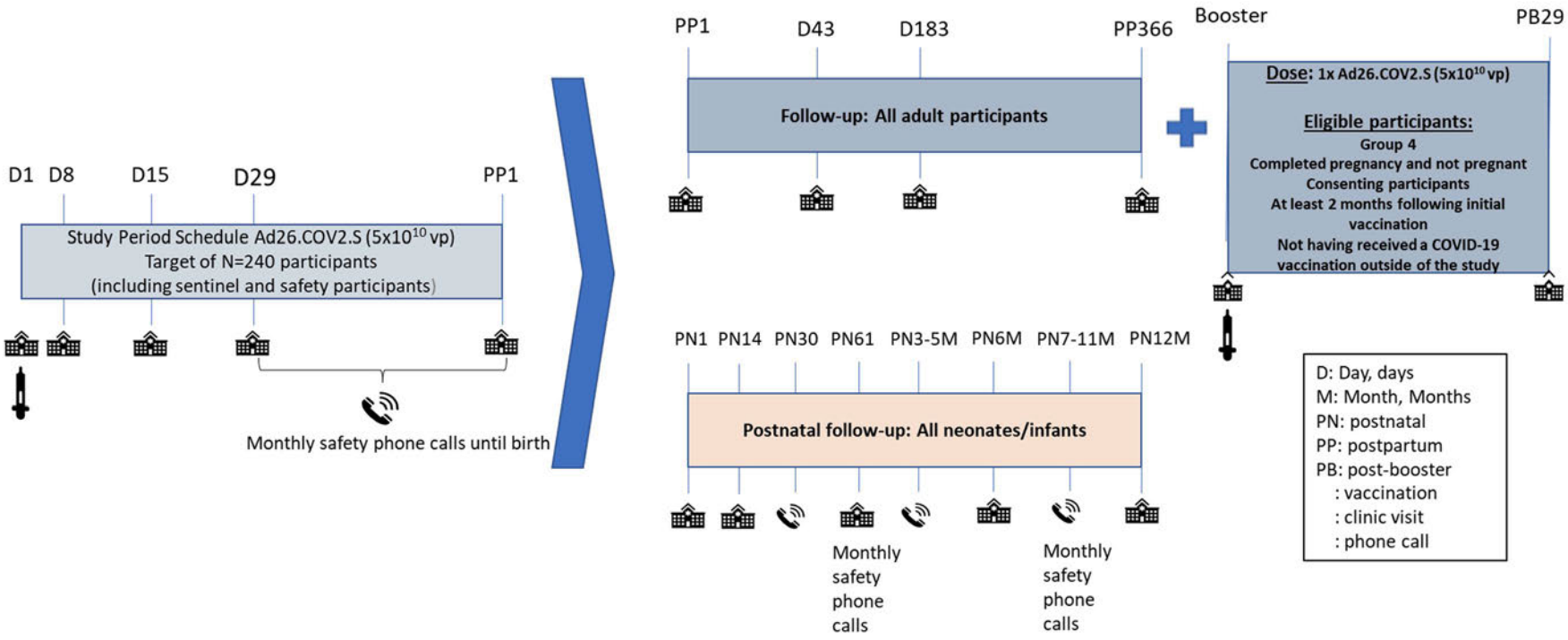
Final Analysis

The final analysis will be performed when all adult participants and all neonates/infants have completed the final visit. Analysis will include safety (AEs, SAEs, and pregnancy outcomes) and immunogenicity data. Booster dose effect will be evaluated on safety and immunogenicity.

Note: Interim analyses may be performed as required or if requested by health authorities.

1.2. Schema

Figure 1: Schematic Overview of the Study



Monthly phone calls are intended for safety follow up and delivery planning, should start after Day 29 and continue periodically until birth.

For the main study, the aim is to recruit at least 40 participants in Groups 1 3 and at least 25 participants in Group 4. All these participants will receive a single dose of Ad26.COV2.S at 5×10^{10} vp.

A booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp will be offered to ongoing, consenting participants who were vaccine naïve at study entry (Group 4, including Sentinel and Safety Cohorts), and who have completed the pregnancy during which they were enrolled in the study, are not pregnant again and have not received another COVID 19 vaccine (eg, national immunization program). If a booster is administered in these participants, the postpartum and booster visits can be combined.

1.3. Schedules of Activities

1.3.1. Adult Participants: Group 4: (Including Sentinel and Safety Cohorts)

Phase[z]	Screening[a]	Study Period					Birth	Follow-up			
Visit[b]	1	2[y]	3	4	5	6-8*	9	10	11	12[ee]	EE[c]
Visit Timing		Dose 1	Dose 1 + 7d	Dose 1 + 14d	Dose 1 + 28d	Monthly phone calls after Dose 1 + 28 days	Pregnancy completion/ termination	PP 1 + 42d	PP 1 + 6 m	PP 1 + 12 m End of Study	Early Exit
Visit Day and (Window)	-28 to 1	D1	D8 (±3d)	D15 (±3d)	D29 (±7d)		PP 1[d] (-1 to +3d)	PP 43 (±14d)	PP 183 (±14d)	PP 366 (±14d)	
Visit Type	Screening	Vaccination	Safety	Safety	Safety Immuno	Phone Call for Safety follow-up	Safety Immuno	Safety Immuno	Safety Immuno	Safety	Early exit
Informed consent (ICF)[e]	●										
Inclusion/exclusion criteria	●	●[1,f]									
Randomization		●[1]									
Demographics [g]	●										
Medical and obstetric history[h]; pre-pregnancy and prestudy meds	●	●[1]									
History of COVID-19 vaccination[bb]	●										
Full physical examination[h]	●	●[1]									●
Obstetric examination[h]	●	●[1]									●
Targeted physical examination[h]			●	●	●		●	●	●	●	
Vital signs including body temperature[aa]	●	●[2]	●	●	●		●	●	●	●	●
Pulse oximetry[i]		●[1]									
Obstetric ultrasound[k]	●	●[1]									●
Nasal swab sample and test for SARS-CoV-2 RNA[l]	●[3]	●[1,4]									
Pre-vaccination symptoms[m]		●[1]									
eCOA training and set-up[n]		●[1]									
Distribution of rulers and thermometers		●									
Distribution of pulse oximeter[o]		●									
Training and distribution: nasal swab kit[n,o]		●									
Symptoms of Infection with Coronavirus-19 (SIC), including highest body temperature measured by the participant (ePROs to be completed by the participant in the eCOA)[p]		●[1]									
Vaccination		●									
30 minutes (60 minutes for the Safety Cohorts) post-vaccination observation[q]		●									
Solicited AE collection[r]		-----*Continuous*-------									

Phase[z]	Screening[a]	Study Period					Birth	Follow-up			
Visit[b]	1	2[y]	3	4	5	6-8*	9	10	11	12[ee]	EE[c]
Visit Timing		Dose 1	Dose 1 + 7d	Dose 1 + 14d	Dose 1 + 28d	Monthly phone calls after Dose 1 + 28 days	Pregnancy completion/ termination	PP 1 + 42d	PP 1 + 6 m	PP 1 + 12 m End of Study	Early Exit
Visit Day and (Window)	-28 to 1	D1	D8 (±3d)	D15 (±3d)	D29 (±7d)		PP 1[d] (-1 to +3d)	PP 43 (±14d)	PP 183 (±14d)	PP 366 (±14d)	
Visit Type	Screening	Vaccination	Safety	Safety	Safety Immuno	Phone Call for Safety follow-up	Safety Immuno	Safety Immuno	Safety Immuno	Safety	Early exit
Investigator's assessment of reactogenicity (participant's diary)			●[5]								
Unsolicited AE collection[r]		----------*Continuous*----------									
Investigator's assessment of reactogenicity (participant's diary)			●[5]								
(Suspected) COVID-19 surveillance (symptom check)[s]		----------*Continuous*----------									
Adverse maternal, fetal outcomes		----------*Continuous*----------									●
AE/SAE/AESI/MAAE recording[t]		----------*Continuous*----------									●
Concomitant medications[u]		----------*Continuous*----------									●
Delivery history[v]							●				
Safety Sampling											
Laboratory assessments, ~ 6.5 mL[j]	●[#] (~6 5 mL)	●[1] (~6 5 mL)	● (~6 5 mL)	●[cc] (~6 5 mL)	●[cc] (~6 5 mL)		● (~6 5 mL)	● (~6 5 mL)			
Whole blood, 12.5 mL[w] Sample will be separated: Plasma sample, 5 mL[w], Serum, 7.5 mL[w]		●[1] (5 mL), (7 5 mL)	● (5 mL), (7 5 mL)		● (5 mL), (7 5 mL)		● (5 mL), (7 5 mL)	● (5 mL), (7 5 mL)			
Other sampling											
Humoral immunogenicity sample (serum)[r]		●[1] (10 mL)			● (10 mL)		● (10 mL)		● (10 mL)		
N-serology sample		●[1] (2 5 mL)			● (2 5 mL)		● (2 5 mL)		● (2 5 mL)		
Colostrum and breast milk sample, mL (optional)[x]							● colostrum 0 5 mL	● breast milk, ≥5 mL			
Vaccination-related biomarker blood sample (PAXgene® tubes, 2.5 mL)		●[1] (2 5 mL)			● (2 5 mL)		● (2 5 mL)				
Cellular immunity (PBMC) blood draw Subset of approximately 25 participants per group, if feasible (optional)[dd]		●[1] (30 mL)			● (30 mL)		● (30 mL)				

Monthly phone calls are intended for safety follow up and delivery planning, should start after Day 29 and continue until birth; #optional laboratory assessments at screening to be done at the discretion of the investigator.

[1] pre dose; [2]pre and post dose; [3]Screening diagnostic tests for current SARS CoV 2 infection can be performed. [4]SARS CoV 2 test is to be repeated pre vaccination on Day 1 only if the test at screening was not done or was done more than 4 days before Day 1 visit. [5] local and systemic solicited AEs should be verified and reported in eDC according to the toxicity

grading scale. if an event is ongoing 7 days post vaccination, the participant should be encouraged to continue filling in the diary until resolution and the rest of the diary may be reviewed again at the next visit.

Footnotes:

a. Screening can be performed within 28 days prior to the study vaccination or on the day of vaccination. If screening is performed on the day of vaccination (recommended), Visit 1 and Visit 2 will coincide on Day 1. In that case, laboratory assessments should only be done once. During the screening visit, at least, all eligibility criteria must be verified prior to randomization and vaccination.

b. If allowed by local regulations: study visits may take place at the participant's home or other location in the event of an ongoing SARS CoV 2 infection performed; visits can be performed by a trained health care professional (HCP) via phone call or a telemedicine contact.

c. For those participants who are unable to continue participation in the study until the end of study visit, but for whom consent is not withdrawn, an early exit visit will be conducted as soon as possible. Participants who wish to withdraw consent from participation in the study will be offered an optional visit for safety follow up. For these participants only the safety assessments of the early exit visit (no blood sampling for immunogenicity) will be performed.

d. Postpartum (PP) 1 refers to the day of pregnancy completion/termination. Visit 9 (PP 1) should occur on the day of pregnancy completion/termination or within 3 days after pregnancy completion/termination. Assessments collected the day before birth can be used, but are preferably collected on the date of birth. If the delivery takes place in a hospital where the site staff does not have privileges, it is expected that medical records are obtained to collect the applicable protocol required safety information. Blood samples are not expected in this case.

e. Signing of the ICF should be done before any study related procedure. The ICF can be signed remotely prior to the Screening Visit. By signing the main ICF, consent is also given to follow up the neonates/infants after birth. Note: Logistics such as downloading of an application to the participant's eDevice, or accessing study materials for enrollment, are not considered a study related procedure.

f. Check clinical status again before study vaccination.

g. Maternal demographic information to be collected at screening includes age, race/ethnicity, and geographical location/residence.

h. Information to be collected for medical and obstetric history, physical examinations and vital signs is specified in Section 8.2. Only relevant medical history (general and obstetric) is to be collected, in particular: history of congenital abnormalities, history of cancer, history of immunodeficiency or conditions treated with immunomodulators, major psychiatric illness, major cardiovascular or lung diseases, history of an allergy to vaccination, ongoing relevant comorbidities per investigator's judgement, history of any comorbidities known to be associated with an increased risk of progression to severe COVID 19 (see Section 10.9) , and history of hepatitis B or hepatitis C infection.

i. Baseline pulse oximetry measurements will be used to estimate the amount of oxygen in the blood, low levels may indicate either SARS CoV 2 infection or an underlying medical condition.

j. For laboratory assessments, including chemistry and hematology assessment of platelet counts see Appendix 2, Table 6 for details. If the data obtained from the laboratory assessment shows abnormal results that are considered clinically significant, then additional blood samples could be taken until normalization of the readings, at the discretion of the investigator. Urine dipstick for protein and glucose will be performed at these visits. If urinalysis results are positive additional work up for pre eclampsia or gestational diabetes may be warranted, but do not preclude inclusion in the study.

k. See Section 8.2.1 for details on obstetric ultrasound. Ultrasound is not required at Visit 2 if screening (Visit 1) ultrasound has been obtained within the past 10 days and there is no other indication for repeating the ultrasound (eg, fetal abnormalities).

l. Diagnostic molecular RT PCR test for SARS CoV 2 infection will be performed at a local laboratory. Test failures or tests that are indeterminate must be repeated. For exploratory endpoint purposes, a molecular RT PCR test for SARS CoV 2 infection may be performed at a central laboratory and will not be reported to the sites. In case of a positive nasal swab result at screening or D1, an exclusion criterion has been met, and samples will not be shipped to central laboratory.

m. The investigator must check for acute illness or body temperature ≥38.0°C/100.4°F at the time of vaccination. If any of these events occur within 24 hours prior to the planned vaccination, the vaccination can be rescheduled in accordance to the protocol's window. For further details refer to Section 5.5.

n. Site staff should provide training to participants to complete the reactogenicity diary using the eCOA on their own eDevice (smartphone or tablet) when feasible, provisioned devices will be available on a limited basis. If a participant is unable to complete the eCOA, a study staff member can collect the information on the participant's behalf as detailed in Section 8.1.2. eCOA set up should be completed before any tests, procedures, to avoid influencing participant responses.

o. On Day 1, all participants will be provided a pulse oximeter (to measure blood oxygen saturation and pulse rate), thermometer and a nasal swab kit to be used in case of a COVID-19 episode (see Section 8.1.2). The pulse oximeter and thermometer for the neonate/infant can also be provided.

p. The SIC questionnaire asks the participant if she had any of the prespecified signs or symptoms of COVID 19 (see Section 10.8 Appendix 8) during the past 24 hours (including highest temperature in the last 24 hours), and (when applicable) to rate the severity. The baseline SIC questionnaire (Visit 2) must be completed prior to vaccine administration.

q. Participants will be closely observed at the site for a minimum of 30 minutes (60 minutes for Sentinels and participants in the Safety Cohort) post vaccination. Sentinels and participants in the Safety Cohort will be vaccinated at least 1 hour apart.
r. Solicited AEs (reactogenicity) and unsolicited AEs will be assessed from time of vaccination until 7 days post vaccination and 28 days post vaccination, respectively.
s. Participants will be asked at least twice a week, through the eCOA, if they have experienced any new symptoms or health concerns that could be related to infection with SARS CoV 2. Refer to Section 1.3.4 Procedures for Participants with (Suspected) COVID 19. In addition, participants will be asked daily within the 30 day time period post vaccination if they have experienced any health concerns related to suspected AESI's, and if so, participants will be advised to contact the study center. Refer to Section 8.3.6 Adverse Events of Special Interest.
t. All SAEs related to study procedures or non investigational sponsor products will be reported from the time a signed and dated ICF is obtained until the end of the study/early withdrawal. MAAE are to be reported for all participants from the moment of the vaccination until 6 months after vaccination. MAAEs leading to study discontinuation which are to be reported until completion of the participant's last study related procedure. New onset of chronic diseases will be collected as part of the MAAEs. See Section 8.3.1 for more details.
u. Refer to Section 6.8 for collection and recording of concomitant therapies.
v. Information to be collected for delivery history.
w. Blood volume will be divided into plasma and serum as described in the laboratory manual and stored for potential coagulation and/or hematology related testing in a central laboratory (see Section 10.2, Appendix 2, Table 7). Unused samples may be used for immunogenicity testing.
x. Approximately 0.5 mL of colostrum should be collected within 1 week postpartum and approximately ≥5 mL of breast milk around PP43. Collection of breast milk and colostrum is optional. Whether the adult participant is breastfeeding, and until when, will be recorded.
y. A booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp will be offered to ongoing, consenting participants who were vaccine naïve at study entry (Group 4, including Sentinel and Safety Cohorts) and who have completed the pregnancy during which they were enrolled in the study, are not pregnant again (as specified in Section 1.3.1.1) under the conditions delineated in Section 6.2. The booster vaccination will be administered not earlier than 2 months after the participant's Ad26.COV2.S vaccination in the study. The Booster Vaccination Visit should preferably coincide with a scheduled visit. If not operationally feasible to coincide with an existing visit, an unscheduled visit may be planned. After the booster vaccination and 28 day post booster visit, participants will continue procedures and visits as in the original SoA.
z. In the situation of a completion/ termination of the pregnancy before the completion of the applicable Study Period visits, PP1 (Visit 9) is to be completed. The participant should then follow the visits scheduled in the 'Study Period' up to Visit 5 (meaning all scheduled visits in this period except for the monthly phone calls Visits 6 8) AND the 'Follow up' section of the schedule of activities (Visits 10 12). Additional guidance if the pregnancy is completed/ terminated before the pre partum visits are completed is provided in Appendix 10.13.
aa. Body temperature will be measured preferably via the oral route, or in accordance with the local standard of care.
bb. History of COVID 19 vaccination (name/manufacturer of the vaccine and date of administration) ≥4 months prior to the study vaccine will be reported in the eCRF.
cc. Laboratory assessments performed at Visits 4 and 5 are optional. If the outcome from the laboratory assessment at Visit 3 show abnormal results that are considered clinically significant, then an on site visit should be performed. In this case, an additional sample should be taken for further assessment 7 days later and until normalization of the readings, at the discretion of the investigator and targeted physical examinations and vital signs should be collected. If the outcome from the laboratory assessment at Visit 3 show normal results, only a safety phone call is required (no physical examinations and vital signs needed).
dd. With only 1 participant in this subset at the time of writing this Protocol Amendment 7, PBMC sampling will be removed from the study as of Protocol Amendment 7 approval.
ee. The Follow up safety assessments for adults at the end of the study visit (no.12) may be performed simultaneously with postnatal (PN 12 months) follow up safety visit (no. 14) as feasible.

AE adverse event; AESI adverse event of special interest; COVID 19 coronavirus disease 2019; d day(s); eCOA electronic clinical outcome assessment; eDC electronic data capture; FU follow up; ICF informed consent form; MAAE medically attended adverse event; m months; PBMC peripheral blood mononuclear cell; PP postpartum; SAE serious adverse event; SIC Symptoms of Infection with Coronavirus 19; SARS CoV 2 severe acute respiratory syndrome coronavirus2.

1.3.1.1. Vaccine Naïve Adult Participants (Group 4, Including Sentinel and Safety Cohorts): Booster Vaccination Visits

Participants who are vaccine naïve at study entry will be offered an (optional) booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp.

The booster will be offered (as outlined in Section 6.2) to:

1. Group 4 participants, including Sentinel and Safety Cohorts,
2. Participants who are ongoing and consenting,
3. Participants who have completed the pregnancy during which they were enrolled in the study, are not pregnant again,
4. Participants have not received another COVID-19 vaccine (eg, national immunization program), and
5. Participant does not meet any criterion per section 7.1 (Discontinuation of Study Vaccination)

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, but are not eligible for a second booster during the study.

1.3.1.2. Vaccine Naïve Adult Participants (Group 4, Including Sentinel and, Safety Cohorts): Booster Vaccination Schedule

Visit #	**Booster Vaccination Visit PB 1**[a]	**PB 1 + 28d**[b]
Visit Timing and Window	**No earlier than Vac1 + 2 months**	**PB 29 (±7d)**
Informed consent[c]	●#	
Vital signs including body temperature[d]	●#	
Pulse oximetry	●#	
Pre-vaccination symptoms[e]	●#	
Targeted physical examination[f]	●#	●
Urine pregnancy test	●#	
Blood sample for humoral immunogenicity (serum), mL[g]	●#10 mL	●10 mL
N-serology[g]	●#2.5 mL	●2.5 mL
Vaccination-related biomarker blood sample (PAXgene® tubes, 2.5 mL)	●#2.5 mL	●2.5 mL
Colostrum (≥0.5 mL)/ Breast milk sample (≥5mL) (optional)[n]	●	●
Clinical laboratory blood sample (whole blood)[g,h]	●#15 mL	●15 mL
Vaccination	●	
30 minutes post-vaccination observation	●	
Solicited AE recording[i]	●	
Investigator's assessment of reactogenicity (participant's diary)		●[i]
Breast milk production changes[p]	●	●
Unsolicited AE recording[i]	●	
(Suspected) COVID-19 surveillance (symptom check)[k]	----------*Continuous*----------	
AE/SAE/MAAE/AESI recording[l]	----------*Continuous*----------	
Concomitant therapies[m]	●[o]	●

Procedure to be completed pre-vaccination

a. The booster vaccination will be administered not earlier than 2 months after completion of the participant's Ad26.COV2.S vaccination in the study and should be administered latest at birth +11 months. The study duration will not be extended. (See Section 6.2).
b. After the Booster Vaccination Visit, participants will resume procedures and visits depicted in Schedule of Activities, Section 1.3.1.
c. Signing of the ICF should be done before any visit related procedures are performed.
d. Body temperature will be measured preferably via the oral route, or in accordance with the local standard of care.
e. Investigator must check for acute illness or body temperature ≥38.0°C/100.4°F at the time of vaccination. If any of these events occur within 24 hours prior to the planned vaccination, the vaccination can be rescheduled as long as this is within the allowed window.
f. Information to be collected for targeted physical examinations and vital signs is specified in Section 8.2
g. For the booster vaccination visit, blood samples should be taken except when the previous sample for assessment of humoral immunogenicity occurred within 5 days of the visit. If a scheduled visit falls within the PB 1 + D28 visit window (±7d), the PB 1 + D28 visit may be combined with the scheduled visit, and any blood samples that are listed in both visits should only be collected once.
h. Whole blood samples (~2mL) will be used for immediate measurement of a platelet count (as part of a complete blood count, if applicable) in a local laboratory or substitute for local laboratory, depending on local feasibility towards turnaround time of sample processing. Serum (4 mL) and plasma (2.5 mL) samples will be derived from the whole blood samples (8 mL and 5 mL, respectively) for retrospective coagulation related testing in a central laboratory in case of a suspected AESI (see Section 10.2, Appendix 2). Unused samples may be used for immunogenicity testing. If a whole blood sample has been taken within 5 days before vaccination, sample collection does not need to be performed before vaccination.
i. Solicited AEs (reactogenicity) and unsolicited AEs will be assessed from time of vaccination until 7 days post vaccination and 28 post vaccination, respectively.

j. If an event is ongoing 7 days post vaccination, the participant should keep filling in the diary until resolution and the diary may be reviewed again at the next visit.
k. Each participant will be asked at least twice a week, through the eCOA, if they have experienced any new symptoms or health concerns that could be related to infection with SARS CoV 2. Refer to Section 1.3.4 Procedures for Participants with (Suspected) COVID 19. In addition, participants will be asked daily within the 30 day time period post vaccination if they have experienced any health concerns related to suspected AESI's, and if so, participants will be advised to contact the study center. Refer to Section 1.3.4 Adverse Events of Special Interest
l. All SAEs related to study procedures or non investigational sponsor products will be reported from the time a signed and dated ICF is obtained until the end of the study/early withdrawal. MAAE are to be reported for all participants from the moment of the vaccination until 6 months after vaccination or end of study duration, whatever comes first, except for MAAEs leading to study discontinuation which are to be reported until completion of the participant's last study related procedure. New onset of chronic diseases will be collected as part of the MAAEs. See Section 8.3.1 for more details.
m. Refer to Section 6.8 for collection and recording of concomitant therapies.
n. Approximately 0.5 mL of colostrum or approximately ≥5 mL of breast milk will be collected if participant is breastfeeding. Collection of breast milk and colostrum is optional.
o. Investigator must check if the participant has received another COVID 19 vaccine outside the study (eg, national immunization program). If so, the participant is not allowed to receive the booster dose
p. Participants will be asked to confirm whether subjective changes in (postpartum) breast milk production (reduction) have been noticed (yes/no) and report changes daily in the eCOA diaries.

AE adverse event; AESI adverse event of special interest; D day(s); ICF informed consent form; MAAE medically attended adverse event; PB post booster; SAE serious adverse event; SARS CoV 2 severe acute respiratory syndrome coronavirus 2.

1.3.2. Adult Participants: Groups 1-3: Previously Vaccinated Participants: Dose 1 Schedule

Phase[y]	Screening[a]	Study period					Birth	Follow-up			
Visit number[b]	1	2	3	4	5	6-8*	9	10	11	12[dd]	EE[c]
Visit Timing		Dose 1	Dose 1 + 7d	Dose 1+ 14d	Dose 1 + 28d	Monthly phone calls after Dose 1 + 28 days	Pregnancy completion/ termination	PP 1 + 42d	PP 1 + 6 m	PP 1 + 12 m End of Study	Early Exit
Visit Day and (Window)	-28 to 1	D1	D8 (±3d)	D15 (±3d)	D29 (±7d)		PP 1[d] (+3d)	PP 43 (±14d)	PP 183 (±14d)	PP 366 (±14d)	
Visit Type	Screening	Vaccination	Safety	Safety	Safety Immuno	Phone Call for Safety follow-up	Safety Immuno	Safety Immuno	Safety Immuno	Safety	Early exit
Informed consent (ICF)[e]	●										
Inclusion/exclusion criteria	●	●[1,f]									
Randomization		●[1]									
Demographics[g]	●										
Medical and obstetric history[h]; pre-pregnancy and prestudy meds	●	●[1]									
History of COVID-19 vaccination[aa]	●										
Full physical examination[h]	●	●[1]									●
Obstetric examination[h]	●	●[1]									●
Targeted physical examination[h]			●	●	●		●	●	●	●	
Vital signs including body temperature[z]	●	●[2]	●	●	●		●	●	●	●	●
Pulse oximetry[i]		●[1]									
Obstetric ultrasound[k]	●	●[1]									●
Nasal swab sample and test for SARS-CoV-2 RNA[l]	●[3]	●[1,4]									
Pre-vaccination symptoms[m]		●[1]									
eCOA training and set-up[n]		●[1]									
Distribution of rulers and thermometers		●									
Distribution of pulse oximeter[o]		●									
Training and distribution: nasal swab kit[n,o]		●									
Symptoms of Infection with Coronavirus-19 (SIC), including highest body temperature measured by the participant (ePROs to be completed by the participant in the eCOA)[p]		●[1]									
Vaccination		●									
30 minutes post-vaccination observation[q]		●									
Solicited AE collection[r]		●	●								
Unsolicited AE collection[r]		●	●	●	●						
Participant reactogenicity diary review by site staff			●[5]								
(Suspected) COVID-19 surveillance (symptom check)[s]		----------*Continuous*----------									
Adverse maternal, fetal outcomes		----------*Continuous*----------									●
AE/SAE/AESI/MAAE recording[t]		----------*Continuous*----------									●

Phase[y]	Screening[a]	Study period					Birth	Follow-up			
Visit number[b]	1	2	3	4	5	6-8*	9	10	11	12[dd]	EE[c]
Visit Timing		Dose 1	Dose 1 + 7d	Dose 1+ 14d	Dose 1 + 28d	Monthly phone calls after Dose 1 + 28 days	Pregnancy completion/ termination	PP 1 + 42d	PP 1 + 6 m	PP 1 + 12 m End of Study	Early Exit
Visit Day and (Window)	-28 to 1	D1	D8 (±3d)	D15 (±3d)	D29 (±7d)		PP 1[d] (+3d)	PP 43 (±14d)	PP 183 (±14d)	PP 366 (±14d)	
Visit Type	Screening	Vaccination	Safety	Safety	Safety Immuno	Phone Call for Safety follow-up	Safety Immuno	Safety Immuno	Safety Immuno	Safety	Early exit
Concomitant medications[u]		----------*Continuous*----------									●
Delivery history[v]							●				
Safety sampling											
Laboratory assessments, ~ 6.5 mL[j]	●[#] (~6 5 mL)	●[1] (~6 5 mL)	● (~6 5 mL)	●[bb] (~6 5 mL)	●[bb] (~6 5 mL)		● (~6 5 mL)	● (~6 5 mL)			
Whole blood, 12.5 mL[w] Sample will be separated: Plasma sample, 5 mL[w] Serum, 7.5 mL[w]		●[1] (5 mL), (7 5 mL)	● (5 mL), (7 5 mL)		● (5 mL), (7 5 mL)		● (5 mL), (7 5 mL)	● (5 mL), (7 5 mL)			
Other sampling											
Humoral immunogenicity sample (serum)[r]		●[1] (10 mL)			● (10 mL)		● (10 mL)		● (10 mL)		
N-serology sample		●[1] (2 5 mL)			● (2 5 mL)		● (2 5 mL)		● (2 5 mL)		
Colostrum and breast milk sample, mL (optional)[x]							● colostrum 0 5 mL	● breast milk, ≥5 mL			
Vaccination-related biomarker blood sample (PAXgene® tubes, 2.5 mL)		●[1] (2 5 mL)			● (2 5 mL)		● (2 5 mL)				
Cellular immunity (PBMC) blood draw Subset of approximately 25 participants per group, if feasible (optional)[cc]		●[1] (30 mL)			● (30 mL)		● (30 mL)				

Monthly phone calls are intended for safety follow up and delivery planning, should start after Day 29 and should continue until birth; #optional laboratory assessments at screening to be done at the discretion of the investigator.

[1] pre dose; [2]pre and post dose; [3]Screening diagnostic tests for current SARS CoV 2 infection will be performed. SARS CoV 2 test is to be repeated pre vaccination on Day 1 only if the test at screening was not done or was done more than 4 days before the Day 1 visit. if an event is ongoing 7 days post vaccination, the participant should be encouraged to continue filling in the diary until resolution and the rest of the diary may be reviewed again at the next visit.

Footnotes:

a. Screening can be performed within 28 days prior to the study vaccination or on the day of vaccination. If screening is performed on the day of vaccination (recommended), Visit 1 and Visit 2 will coincide on Day 1. In that case, assessments should only be done once. During the screening, at least, all eligibility criteria must be verified prior to randomization and vaccination.

b. If allowed by local regulations: study visits may take place at the participant's home or other location in the event of an ongoing SARS CoV 2 infection; visits can be performed by a phone call or a telemedicine contact; except for vaccination visits, scheduled assessments may be performed by a trained HCP.

c. For those participants who are unable to continue participation until the end of study visit, but for whom consent is not withdrawn, an early exit visit will be conducted as soon as possible. Participants who wish to withdraw consent from participation in the study will be offered an optional visit for safety follow up. For these participants only the safety assessments of the early exit visit (no blood sampling for immunogenicity) will be performed.

d. Postpartum (PP) 1 refers to the day of pregnancy completion/termination. Visit 9 (PP 1) should occur on the day of pregnancy completion/termination or within 3 days after pregnancy completion/termination. Assessments collected the day before birth can be used but are preferably collected on the date of birth (for example in case of pre planned ceasarian). If the delivery takes place in a hospital where the site staff does not have privileges, it is expected that medical records are collected to collect the applicable protocol required information. Blood samples are not expected in this case.
e. Signing of the ICF should be done before any study related procedure. The ICF can be signed remotely prior to the Screening Visit. Downloading of an application to the participant's eDevice, to access materials for enrollment and study information, is not considered a study related procedure. By signing the main ICF, consent is also given to follow up the neonates/infants after birth.
f. Check clinical status again before study vaccination.
g. Maternal demographic information to be collected at screening includes age, race/ethnicity, and geographical location/residence.
h. Information to be collected for medical and obstetric history, physical examinations and vital signs is specified in Section 8.2. Only relevant medical history (general and obstetric) is to be collected, in particular: history of congenital abnormalities, history of cancer, history of immunodeficiency or conditions treated with immunomodulators, major psychiatric illness, major cardiovascular or lung diseases, history of an allergy to vaccination, ongoing relevant comorbidities per investigator's judgement, history of any comorbidities known to be associated with an increased risk of progression to severe COVID 19 (see Section 10.9) , and history of hepatitis B or hepatitis C infection.
i. Baseline pulse oximetry measurements will be used to estimate the amount of oxygen in the blood, low levels may indicate either SARS CoV 2 infection or an underlying medical condition.
j. For laboratory assessments, including chemistry and hematology assessment of platelet counts (to be done locally within 72 hours of blood collection) see Appendix 2, Table 6 for details. If the outcome from the laboratory assessment shows abnormal results that are considered clinically significant, then additional blood samples could be taken until normalization of the readings, at the discretion of the investigator. Urine dipstick for protein and glucose will be performed at these visits. If urinalysis results are positive additional work up for pre eclampsia or gestational diabetes may be warranted, but do not preclude inclusion in the study.
k. See Section 8.2.1 for details on obstetric ultrasound. Ultrasound is not required at Visit 2 if screening (Visit 1) ultrasound has been obtained within the past 10 days and there is no other indication for repeating the ultrasound (eg, fetal abnormalities).
l. Diagnostic molecular RT PCR test for SARS CoV 2 infection will be performed at a local laboratory. For exploratory endpoint purposes, a molecular RT PCR test for SARS CoV 2 infection may be performed at a central laboratory and will not be reported to the sites. Test failures or tests that are indeterminate must be repeated. In case of a positive nasal swab result at screening or D1, an exclusion criterion has been met, and samples will not be shipped to central laboratory.
m. The investigator must check for acute illness or body temperature ≥38.0°C/100.4ºF at the time of vaccination. If any of these events occur within 24 hours prior to the planned vaccination, the vaccination can be rescheduled in accordance to the protocol's window. For further details refer to Section 5.5.
n. Participants will complete the reactogenicity diary using the eCOA on their own eDevice (smartphone or tablet) when feasible, also provisioned devices will be available on a limited basis. If a participant is unable to complete the eCOA, a study staff member can collect the information on the participant's behalf as detailed in Section 8.1.2. All eCOA assessment should be conducted/completed before any tests, procedures, or other consultations, in order to avoid influencing participant responses.
o. At Baseline, all participants will be provided a pulse oximeter (to measure blood oxygen saturation and pulse rate), thermometer and a nasal swab kit to be used in case of a COVID 19 episode (see Section 8.1.2). The pulse oximeter and thermometer for the neonate/infant can also be provided.
p. The SIC questionnaire asks the participant if she had any of the prespecified signs or symptoms of COVID 19 (see Section 10.8 Appendix 8) during the past 24 hours (including highest temperature in the last 24 hours), and (when applicable) to rate the severity. The baseline SIC questionnaire (Visit 2) must be completed prior to vaccine administration.
q. Participants will be closely observed at the site for a minimum of 30 minutes post vaccination.
r. Solicited AEs (reactogenicity) and unsolicited AEs will be assessed from time of vaccination until 7 days post vaccination and 28 post vaccination, respectively.
s. Participant will be asked at least twice a week, through the eCOA, if they have experienced any new symptoms or health concerns that could be related to infection with SARS CoV 2. Refer to Section 1.3.4 Procedures for Participants with (Suspected) COVID 19. In addition, participants will be asked daily within the 30 day time period post vaccination if they have experienced any health concerns related to suspected AESI's, and if so, participants will be advised to contact the study center. Refer to Section 8.3.6 Adverse Events of Special Interest.
t. All SAEs related to study procedures or non investigational sponsor products will be reported from the time a signed and dated ICF is obtained until the end of the study/early withdrawal. MAAE are to be reported for all participants from the moment of the vaccination until 6 months after vaccination. MAAEs leading to study discontinuation which are to be reported until completion of the participant's last study related procedure. New onset of chronic diseases will be collected as part of the MAAEs. See Section 8.3.1 for more details.
u. Refer to Section 6.8 for collection and recording of concomitant therapies
v. Information to be collected for delivery history.

w. Blood volume will be divided into plasma and serum as described in the laboratory manual and stored for potential coagulation and/or hematology related testing in a central laboratory (see Section 10.2, Appendix 2, Table 7). Unused samples may be used for immunogenicity testing.
x. Approximately 0.5 mL of colostrum should be collected within 1 week postpartum and approximately ≥5 mL of breast milk around PP43. Collection of breast milk and colostrum is optional. Whether the adult participant is breastfeeding, and until when, will be recorded.
y. In the situation of a completion/ termination of the pregnancy before the completion of the applicable Study Period visits, PP1 (Visit 9) is to be completed. The participant should then follow the visits scheduled in the 'Study Period' up to visit 5 (meaning all scheduled visits in this period except for the monthly phone calls Visits 6 8) AND the 'Follow up' section of the schedule of activities (Visits 10 12). Additional guidance if the pregnancy is completed/ terminated before the pre partum visits are completed is provided in Appendix 10.13.
z. Body temperature will be measured preferably via the oral route, or in accordance with the local standard of care.
aa. Any history of COVID 19 vaccination (name/manufacturer of the vaccine and date of administration) ≥4 months prior to the study will be reported in the eCRF.
bb. Laboratory assessments performed at Visits 4 and 5 are optional. If the outcome from the laboratory assessment at Visit 3 show abnormal results that are considered clinically significant, then an on site visit should be performed. In this case, an additional sample should be taken for further assessment 7 days later and until normalization of the readings, at the discretion of the investigator and targeted physical examinations and vital signs should be collected. If the outcome from the laboratory assessment at Visit 3 show normal results, only a safety phone call is required (no physical examinations and vital signs needed).
cc. With only 1 participant in this subset at the time of writing this Protocol Amendment 7, PBMC sampling will be removed from the study as of Protocol Amendment 7 approval.
dd. The Follow up safety assessments for adults at the end of the study visit (no.12) may be performed simultaneously with postnatal (PN 12 months) follow up safety visit (no. 14) as feasible.

AE adverse event; AESI adverse event of special interest; COVID 19 coronavirus disease 2019; d day(s); eCOA electronic clinical outcome assessment; FU follow up; ICF informed consent form; MAAE medically attended adverse event; m months; PBMC peripheral blood mononuclear cell; PP postpartum; SAE serious adverse event; SIC Symptoms of Infection with Coronavirus 19; SARS CoV 2 severe acute respiratory syndrome coronavirus.

1.3.3. Neonate/Infants Schedule

Phase	Birth	Postnatal (PN) Follow-up							
Visit #[b]	**1**	**2**	**3**	**4**	**5-7**	**8**	**9-13**	**14[u]**	**EE**
Visit Timing	**Pregnancy Completion (Birth)**	**2 Weeks of Age**	**1 mo**	**2 mo**	**3 to 5 mo**	**6 mo[c,t]**	**7 to 11 mo**	**12 mo[t] End of Study**	**Early Exit[d]**
Visit Day and (Window)	**PN 1 (+3d)[a]**	**PN 14 (± 7 d)**	**PN 30 (± 7 d)**	**PN 61 (± 7 d)**	**PN3 mo to 5 mo (±7 d)**	**PN 6 mo (±14d)**	**PN 7 to 11 mo (±7 d)**	**PN 12 mo (±14d)**	
Visit Type	Safety and Immuno	Safety	Phone Call for Safety follow up	Safety and Immuno	Monthly Phone Call for Safety follow up	Safety and Immuno	Monthly Phone Call for Safety follow up	Safety	Early Exit
Birth outcome	●								
Neonatal medical history[e]	●								
Adverse neonatal outcome	●								
SAE recording[f]	*Continuous*								
MAAE recording[g]	*Continuous*								
Concomitant medication[h]	*Continuous*								
Physical examination[i]	●	●		●		●		●	●
Vital signs and body temperature[j]	●	●		●		●		●	●
Apgar[k]	●								
Blood collection(humoral immunogenicity)[l]	●cord blood only (15 mL)			●3.5mL		●3.5mL			
Vaccination related biomarker blood sample (PAXgene® tubes)	●cord blood only (2.5mL)								
Pulse oximetry by site staff[m]	●1								
Distribution of thermometer and pulse oximeter[n]	●1								
eCOA training and set up[p]	●1								
PedSIC including body temperature measured by the parent/caregiver. eObsRO to be entered by the parent(s) /caregiver(s) in the eCOA[q]	●								
ASQ 3[r]				●		●		●	●[ss]
(Suspected) COVID 19 surveillance (Pediatric Symptom Check)[o]	*Continuous*								

Note: "Participant" refers to neonate or infant in this section. 1. Can be completed the day before birth.

Footnotes:

a. Postnatal (PN) 1 refers to the day of birth. If the delivery takes place in a hospital where the site staff does not have privileges, it is expected that medical records are collected to collect the applicable protocol required information. Blood samples are not expected in this case.

b. If allowed by local regulations: study visits may take place at the participant's home or other location in the event of ongoing SARS CoV 2 transmission in the area of the participant. ; visits can be performed by a phone call or a telemedicine contact. Scheduled assessments may also be performed by a trained HCP.
c. At approximately 6 months of age or 28 days after the last routine primary vaccination (dependent on the local primary vaccinations schedule)
d. For those participants who are unable to continue participation in the study up to Visit 14, but for whom consent is not withdrawn, an early exit visit will be conducted as soon as possible. Parent(s)/caregiver(s) who wish to withdraw consent from participation of the participant in the study will be offered an optional visit for safety follow up for the participant. This includes the safety assessments of the early exit visit. For these participants only the safety assessments of the early exit visit (no blood sampling for immunogenicity) will be performed.
e. Refer to Section 8.2.6 for assessments to be performed as part of neonatal medical history.
f. All SAEs related to study procedures or non investigational sponsor products will be reported from birth until the end of the study/early withdrawal. All other SAEs are to be reported from the moment of birth until completion of the participant's last study related procedure.
g. MAAE are to be reported for all participants from the moment of birth until 6 months of age, except for MAAEs leading to study discontinuation, are to be reported from birth until the participant's last study related procedure. New onset of chronic diseases will be collected as part of the MAAEs
h. Refer to Section 6.8 for collection and recording of concomitant therapies associated with SAEs and MAAEs.
i. Refer to Section 8.2.1 for assessments to be performed.
j. Heart rate, respiratory rate, and body temperature. Vital signs measurements are recommended before blood sampling. Body temperatures will be measured according to the local standard of care (actual routes to be recorded in the eCRF).
k. The overall Apgar score is required to be documented in the eDC. The Apgar score comprises of the parameters: color, heart rate, reflexes, muscle tone, and respiration that could be entered in the eDC if available (See Section 8.2.1).
l. Blood sample for humoral immunity also includes sample for previous sero confirmation of SARS CoV 2 infection (antibody).
m. Baseline pulse oximetry measurements will be used to estimate the amount of oxygen in the blood, with low levels indicative either of SARS CoV 2 infection or an underlying medical condition.
n. If not given on Day 1, parent(s)/caregiver(s) will be provided with a pulse oximeter (to measure blood oxygen saturation and pulse rate) and thermometer no later than Visit PN 1 (to be used during a COVID 19 episode in the neonate/infant [see Section 1.3.5]).
o. If a participant develops (suspected) COVID 19, refer to Section 1.3.5. Each parent/caregiver will be asked at least twice a week, through the eCOA, if the participant has experienced any new symptoms or health concerns that could be related to infection with SARS CoV 2. Sites should reach out to a parent/caregiver if the parent/caregiver fails to complete the surveillance question upon any of these reminders. The questionnaire will be accessible on the eCOA platform in between scheduled reminders and parent(s)/caregiver(s) will be encouraged to answer the surveillance question in the eCOA as soon as possible after the onset of COVID 19 like symptoms. Every effort will be made to document the status of all participants that are lost to follow up due to the parent/caregiver not completing the eCOA and for whom hospitalization has not been recorded.
p. eCOA set up should be done by site staff according to PN1 visit window. Actual training can be completed remotely by the parent(s)/caregiver(s); sites should ensure that parents perform this training no later than on PN14. Parent(s)/caregiver(s) will complete the eCOA using an application on their own eDevice (smartphone or tablet) if their device is compatible with the application. All eCOA assessments should be conducted/completed before any tests, procedures, or other consultations to prevent influencing participant responses. If a parent/caregiver is unable to complete the eCOA, a study staff member can collect information on the participant's behalf as detailed in Section 8.1.2.
q. The PedSIC questionnaire asks the parent(s)/caregiver(s) if their neonate/infant had any of the prespecified signs or symptoms of COVID 19 during the past 24 hours (including highest temperature in the last 24 hours), and (when applicable) to rate the severity.
r. Neurodevelopmental status will be measured using the Ages & Stages Questionnaire, 3rd edition (ASQ 3) as outlined in Section 8.2.6.
s. If Early Exit occurs when the infant is 2, 6 or 12 months old.
t. The PN 6 mo and PN 12 mo visit may coincide with the PP 6 m and PP 12 m, if feasible to respect the visit windows.
u. The Follow up safety assessments for adults at the end of the study visit (no.12) may be performed simultaneously with postnatal (PN 12 months) follow up safety visit (no. 14) as feasible.

d days; eCOA electronic clinical outcome assessment; eDC electronic data capture; mo months of age; ObsRO observer reported outcomes; PedSIC Pediatric Symptoms of Infection with Coronavirus 19; PN postnatal; SAE serious adverse event.

1.3.4. Procedures for Adult Participants With (Suspected) COVID-19

Procedures	Study Period				
Timing relative to onset of signs and symptoms	COVID-19 Day 1-2	COVID-19 Day 3-5[b]		7-day cycle to be repeated[c,d,e,f]	COVID-19 Day 29 (±7 d)[g,h]
		Part 1	Part 2[c]		
Location	Home[l]	Site[j,]	Site[j,k]	Site or Home[k]	Site or Home[j,k]
Participant to contact study site with any health concerns/participant notifies the site of becoming aware of a positive RT PCR test	●				
Site to contact participant if COVID 19 signs or symptoms are recorded in eCOA	●				
Confirmation of suspected COVID 19 using prespecified criteria	●[l]	●[m]			
Nasal swab sample (collected by the participant at home)[n]	●[o]			●	
Nasal swab sample (collected by qualified study staff)		●[p]			
Humoral immunity (serum)			●10 mL		●10 mL,[q]
Biomarker blood sample (PAXgene® tubes, whole blood)			●2.5 mL		●2.5 mL
In case of signs and symptoms: Symptoms of Infection with Coronavirus 19 (SIC), including highest body temperature over the last 24 hours measured by the participant (ePROs to be completed by the participant in the eCOA)[r]	*Daily*				●[s]
In case of no signs or symptoms: (Suspected) COVID 19 surveillance (symptom check)	*At least twice a week*				●
Vital signs[t]		●			●
Targeted physical examination		●			●
Pulse oximetry by site staff		●			●
In case of signs and symptoms: Pulse oximetry by the participant (ePRO) to be completed by the participant in the eCOA)[u]	●[o]	*3 times a day*			
Medical history (including recent flu or pneumococcal vaccination) and description of COVID 19 episode (collected by interview with the participant)			●		●
Concomitant therapies associated with COVID 19	*Continuous*				
Study site personnel to contact participant	At least *weekly*				

a. Note: "Participant" refers to adult participants, either pregnant or postpartum
b. The visit at COVID 19 Day 3 5 should be scheduled 2 to 4 days after symptoms onset/positive RT PCR test from outside the study.
c. Only applicable for participants that meet the prespecified criteria for suspected COVID 19 (see Section 8.1.2.4) on COVID 19 Day 1 2 and COVID 19 Day 3 5.
d. Participant should be encouraged by the site to have nasal swabs collected as indicated in the Schedule of Activities. If the participant is unable or unwilling to have all samples collected as requested, the participant should still complete the other COVID 19 assessments, including the visit at COVID 19 Day 29.
e. If the nasal swabs collected on COVID 19 Day 1 2 and COVID 19 Day 3 5 are negative for SARS CoV 2, the participant will not undertake any further COVID 19 procedures and will fall back to the default Schedule of Activities in Section 1.3.1 or Section 1.3.2 until the end of the study/early withdrawal.

f. Participants should undertake the COVID 19 procedures until 14 days after symptom onset (COVID 19 Day 15) or until resolution of the COVID 19 episode, whichever comes last. Resolution of a COVID 19 episode is defined as having 2 consecutive SARS CoV 2 negative nasal swabs and 2 consecutive days with no COVID 19 related signs or symptoms. Once past COVID 19 Day 15, participants should stop the collection of nasal swabs samples as soon as 2 consecutive nasal samples are SARS CoV 2 negative, but (if still symptomatic at that time) should continue completing the ePROs (including SIC, body temperature, and pulse oximetry) in the eCOA until 2 consecutive days with no COVID 19 related signs or symptoms.
g. Only applicable for participants that have at least 1 SARS CoV 2 positive nasal swab collected on COVID 19 Day 1 2 or Day 3 5. COVID 19 Day 29 should still be performed even if the nasal swab results are still pending.
h. The visit on COVID 19 Day 29 can be combined with a regular study visit if within the applicable visit windows.
i. The COVID 19 Day 1 2 nasal swab can be collected at the study site (or hospital or other location, if needed), if preferred by the participant.
j. All COVID 19 Day 3 5 and Day 29 assessments may be performed by a trained HCP at the participant's home, if allowed per local regulations.
k. If a participant has a positive test result for SARS CoV 2 infection and/or depending on the medical status of the participant. If necessary, study site personnel or a trained HCP may visit the participant at home (or at the hospital or other location, if needed), if allowed by local regulations. Under these circumstances, the participant will be contacted by the site at least once per week.
l. In case of COVID 19 like symptoms, based on the information collected through the SIC, the site will reach out to the participant at the latest on COVID 19 Day 2 (the day after the day of symptom onset) to assess whether the reported signs and symptoms qualify as a suspected COVID 19 episode using prespecified criteria (Section 8.1.2.4). As several of the prespecified criteria for suspected COVID 19 overlap with vaccine related reactogenicity, investigators' clinical judgement is required to exclude vaccine related events when assessing suspected COVID 19. In case the participant would actively reach out to the site already on COVID 19 Day 1, the site should already make a first assessment on COVID 19 Day 1 to check whether the reported signs and symptoms qualify as a suspected COVID 19 episode using prespecified criteria (Section 8.1.2.4).
m. In case of COVID 19 like symptoms, the site will interview the participant to assess whether the reported signs and symptoms still qualify as a suspected COVID 19 episode using prespecified criteria (Section 8.1.2.4).
n. A nasal swab should be collected from the participant at home (using available material for home swabs provided by the study staff) as soon as the prespecified criteria for suspected COVID 19 are met and preferably on the day of symptom onset or the day thereafter (COVID 19 Day 1 2). The sample collected on COVID 19 Day 1 2 should be transferred to the study site, as arranged by the study site, as soon as possible after collection, preferably within 3 days. Nasal swabs should also be collected once every 7 days until 14 days after symptoms onset (COVID 19 Day 15) or until resolution of the COVID 19 episode, whichever comes last. These samples should be transferred to the study site, as arranged by the study site, within 3 days after collection. Details are provided in the laboratory manual. If the participant requires assistance, a trained HCP can help the participant to collect the nasal swabs. If 2 consecutive nasal swabs negative for SARS CoV 2 are not available due to operational reasons (eg, delays in results availability), participants may cease collection of nasal swabs at the COVID 19 Day 29 visit, provided they have 2 consecutive days with no COVID 19 related signs and symptoms. In these cases, participants may be asked to resume sample collection if nasal sample results once available do not present with 2 consecutive negative swabs for SARS CoV 2. All nasal swabs will be tested by a local laboratory for case management.
o. The nasal swab should be collected and pulse oximetry (3 times a day), should be started as soon as possible after it has been confirmed that the prespecified criteria for suspected COVID 19 (Section 8.1.2.4) are met. On the first day the participant experiences signs/symptoms, the participant should record at least 1 of the 3 pulse oximetry readings in the last 24 hours in the eCOA.
p. Nasal swabs will be tested by a local laboratory for case management. For participants with suspected COVID 19, confirmation of SARS CoV 2 infection by RT PCR or other molecular diagnostic test at a central laboratory may be used for the analysis of the case definition.
q. Blood sample for humoral immunity may include a sample for sero confirmation of previous SARS CoV 2 infection (antibody).
r. Participants should complete the (suspected) COVID 19 surveillance (symptom check) in the eCOA. In case of COVID 19 like signs and symptoms, participants should be encouraged by the site to complete the SIC daily, preferably in the evening around the same time each day, starting on the first day they experience symptoms. Sites should remind the participant to complete the SIC, unless special circumstances occur such as hospitalization or ventilation, in which case the reason for not completing the SIC should be recorded by site staff in the clinical database. If signs and symptoms are still ongoing on COVID 19 Day 3 5, collection of SIC will be continued until at least 14 days after symptoms onset unless both COVID 19 Day 1 2 and COVID 19 Day 3 5 nasal swabs are negative. If either of the swabs are positive or the result is unknown AND the participant is beyond 14 days after onset of symptoms, the SIC can be stopped after 2 days without signs and symptoms.
If a participant is unable to complete the eCOA, a study staff member or a caregiver can collect information on the participant's behalf as detailed in Section 8.1.2.1.
Participant should measure body temperature daily (oral route preferred, or in accordance with the local standard of care) and record the highest temperature in the last 24 hours.
s. If the participant does not have symptoms at that time, he/she will only need to complete the (suspected) COVID 19 surveillance (symptom check) in the eCOA.

t. Includes measurement of vital signs (preferably supine systolic and diastolic blood pressure, heart rate, and respiratory rate [after at least 5 minutes rest] and body temperature). It is recommended that vital signs are measured before collection of nasal swabs and blood draws.
u. In case of COVID 19 like symptoms, the participant will be asked to measure blood oxygen saturation and pulse rate at home 3 times a day (preferably in the morning, at lunch time, and in the evening). The results will be recorded by the participant in the eCOA.

Upon closure of the COVID 19 episode and procedures, all participants will fall back to the default Schedule of Activities. Closure of a COVID 19 episode should occur at the last study visit. If the episode is ongoing, it should be marked as such in the eCRF. The episode will be followed by the investigator until resolution or until a clinically stable condition is reached, and the outcome recorded in the participant's medical chart.

COVID 19 coronavirus disease 2019; eCOA electronic clinical outcome assessment; RNA ribonucleic acid; SARS CoV 2 severe acute respiratory syndrome coronavirus 2; SIC Symptoms of Infection with Coronavirus 19.

1.3.5. Procedures for Neonates and Infants With (Suspected) COVID-19

Procedures	Study Period			
Timing relative to onset of signs and symptoms or positive RT-PCR test results from outside the study	**COVID-19 Day 1-3**	**COVID-19 Day 4-7*[b,c,d]**	**7-day cycle to be repeated[c,d,f]**	**COVID-19 Day 29 (±7 d)[c,g]**
Location	**Site or Home[h]**	**Site or Home[h]**	**Site or Home[h]**	**Site or Home[h]**
Parent(s)/caregiver(s) to contact study site with any health concerns/day parent(s)/ caregivers becoming aware of a positive RT PCR test from neonate/infant or from a close relative	●			
Site to contact parent(s)/caregiver(s) if COVID 19 signs or symptoms are recorded in eCOA	●			
Confirmation of suspected COVID 19 using prespecified criteria	●[j]			
Nasal swab sample (collected by qualified study staff)[h,j]	●[m]	●	●	
Humoral immunity (serum)[o]		●2.5mL		●2.5mL
In case of signs and symptoms: Pediatric Symptoms of Infection with Coronavirus 19 (PedSIC), including highest body temperature over the last 24 hours measured by the parent(s)/caregiver(s) (ObsROs to be completed by the parent(s)/caregiver(s) in the eCOA)[l]	*Daily*			●[m]
In case of no signs or symptoms: (Suspected) COVID 19 surveillance (symptom check)	*At least twice a week*			●
Vital signs[n]		●		●
Targeted physical examination		●		●
Pulse oximetry by site staff		●		●
In case of signs and symptoms: Pulse oximetry by the parent(s)/caregiver(s) (ObsRO to be completed by the parent(s)/caregiver(s) in the eCOA)[o]	●[m]	*3 times a day*		
Medical history (including recent flu or pneumococcal vaccination) and description of COVID 19 episode (collected by interview with the parent(s)/caregiver(s))		●		●
Concomitant therapies associated with COVID 19	*Continuous*			
Study site personnel to contact parent(s)/caregiver(s)	At least *weekly*			

*Neonates/infants with a positive test from outside the study context will immediately proceed with the Day 4 7 procedures.

a. Note: "Participant" refers to neonate or infant in this section.
b. The visit at COVID 19 Day 4 7 should be scheduled 1 to 6 days after signs or symptoms onset and a positive RT PCR test from COVID 19 Day 1 3 or positive RT PCR test from outside the study.
c. Only applicable for participants that have a SARS CoV 2 positive nasal swab from COVID 19 Day 1 to 3 or from outside the study (Section 8.1.2.5 and Section 8.1.2.6).

d. Parent(s)/caregiver(s) should be encouraged by the site to have nasal swabs collected from participants (by qualified study staff/HCP) as indicated in the Schedule of Activities. If the parent/caregiver or participant is unable or unwilling to have all samples collected as requested, the participant should still complete the other COVID 19 assessments, including the visit at COVID 19 Day 29.
e. If the nasal swab collected on COVID 19 Day 1 to 3 is negative for SARS CoV 2, the participant will not undertake any further COVID 19 procedures and will fall back to the default Schedule of Activities in Section 1.3.3.
f. Participants should undertake the COVID 19 procedures until 14 days after signs or symptom onset/positive molecular test from outside the study (COVID 19 Day 15) or until resolution of the COVID 19 episode, whichever comes last. Resolution of a COVID 19 episode is defined as having 1 SARS CoV 2 negative nasal swab and 2 consecutive days with no COVID 19 related signs or symptoms. Once past COVID 19 Day 15, collection of nasal swab samples from participants should be stopped as soon as 1 nasal swab is SARS CoV 2 negative, but (if still symptomatic at that time) the participant or parent(s)/caregiver(s) should continue completing the ObsROs (including SIC, body temperature, and pulse oximetry) in the eCOA until 2 consecutive days with no COVID 19 related signs or symptoms.
g. The visit on COVID 19 Day 29 can be combined with a regular study visit if within the applicable visit windows.
h. If allowed by local or institutional regulations, if a participant has a positive test result for SARS CoV.2 infection and/or depending on the medical status of the participant, the participant may be requested not to visit the study site but to stay at home; if necessary, study site personnel may visit the participant at home. Under these circumstances, the participant will be contacted by the site staff at least once per week to follow up on the participant's condition. The site staff or HCP visiting the participant at home will use personal protective equipment. A nasal swab should be collected from the participant by qualified study staff as soon as the prespecified criteria for suspected COVID 19 are met and, in case of COVID 19 like symptoms, preferably on the day of symptom onset or in the 2 days thereafter (COVID 19 Day 1 3). In case of a positive RT PCR sample, nasal swabs should also be collected once every 7 days until 14 days after signs and symptoms onset/positive RT PCR test from outside the study (COVID 19 Day 15, if applicable) or until resolution of the COVID 19 episode, whichever comes last. Participants that have a nasal swab taken at Day 1 3 do not need to repeat the swab at Day 4 7, but go straight into the 7 day cycle. If the nasal swab (Day 1 3, Day 4 7, 7 day cycle) is collected at the participant's home, study staff should arrange transfer of the sample to the study site as soon as possible after collection (and within the timelines specified in Section 8.1.2.2). Details are provided in the laboratory manual.
i. The nasal swab should be collected and pulse oximetry (3 times a day), should be started (if not started already) as soon as possible after it has been confirmed that the prespecified criteria for suspected COVID 19 (Section 8.1.2.5 and 8.1.2.6) are met. On the first day the participant experiences signs/symptoms, the participant should record at least 1 of the 3 pulse oximetry readings in the last 24 hours in the eCOA.
j. All nasal swabs will be tested by a local laboratory for case management and remaining aliquots will be sent to the central laboratory. For participants with suspected COVID 19 after a positive local test, confirmation of SARS CoV 2 infection by a central laboratory will be used for the analysis of the case definition. Nasal swabs may also be tested for the presence of other respiratory pathogens using a broad respiratory pathogens panel.
k. Blood sample for humoral immunity may include sero confirmation of previous SARS CoV 2 infection (antibody).
l. Parent(s)/caregiver(s) should complete the (suspected) COVID 19 surveillance (symptom check). In case of COVID 19 like signs and symptoms, parent(s)/caregiver(s) should be encouraged by the site to complete the PedSIC daily, preferably in the evening around the same time each day, starting on the first day the participant experiences signs or symptoms. Sites should remind the parent/caregiver to complete the PedSIC, unless special circumstances occur such as hospitalization or ventilation, in which case the reason for not completing the PedSIC should be recorded by site staff in the clinical database. If a parent/caregiver is unable to complete the eCOA, a study staff member can collect information on the parent's/caregiver's behalf as detailed in Section 8.1.2.2.
   Parent(s)/caregiver(s) should measure body temperature daily (in accordance with the local standard of care) and record the highest temperature in the last 24 hours.
m. If the participant does not have symptoms at that time, the parent(s)/caregiver(s) will only need to complete the (suspected) COVID 19 surveillance (symptom check).
n. Includes measurement heart rate, respiratory rate [after at least 5 minutes rest, if feasible] and body temperature. It is recommended that vital signs are measured before collection of nasal swabs and blood draws.
o. In case of COVID 19 like signs or symptoms, the parent(s)/caregiver(s) will be asked to measure blood oxygen saturation and pulse rate at home 3 times a day (preferably in the morning, at lunch time, and in the evening). The results will be recorded by the parent(s)/caregiver(s) in the eCOA. On the first day the participant experiences signs/symptoms, the participant should record at least 1 of the 3 pulse oximetry readings in the last 24 hours in the eCOA.

Upon closure of the COVID 19 episode and procedures, all participants will fall back to the default Schedule of Activities. Closure of a COVID 19 episode should occur at the last study visit. If the episode is ongoing, it should be marked as such in the eCRF. The episode will be followed by the investigator until resolution or until a clinically stable condition is reached, and the outcome recorded in the participant's medical chart.

COVID 19 coronavirus disease 2019; eCOA electronic clinical outcome assessment; ObsRO observer reported outcomes; RNA ribonucleic acid; SARS CoV 2 severe acute respiratory syndrome coronavirus 2; PedSIC Pediatric Symptoms of Infection with Coronavirus 19.

Note: Testing is recommended for all neonates born to mothers with confirmed COVID 19 at the time of birth, regardless of whether there are signs of infection in the neonate (see Section 8.1.2).

1.3.6. Participants with a Suspected AESI - Pregnant Participants

The medical management of thrombotic events with thrombocytopenia is different from the management of isolated thromboembolic diseases. Study site personnel and/or treating physicians should follow available guidelines for treatment of thrombotic thrombocytopenia (eg, from the American Society of Hematology 2021, British Society of Haematology - Expert Haematology Panel 2021, and the CDC 2021).

The use of heparin may be harmful and alternative treatments may be needed. Consultation with a hematologist is strongly recommended. Management of the participant should not be delayed by decision making of the AESI Adjudication Committee.

In the event of a suspected post-vaccination thrombotic event, thrombocytopenia, or TTS, additional laboratory assessments might be needed to declare a true case, including (but not limited to) platelet count and anti-PF4 tests. Additional blood samples should be collected for central laboratory testing as detailed below. However, results of central laboratory testing may not be available to guide immediate treatment decisions.

Procedures	**Study Period**	
Timing relative to onset of suspected AESI	**AESI Day 1[a]**	**AESI Day 29[b]**
Visit Window		**±7 d**
Site to report suspected AESI[c]		
Clinical laboratory blood sample (whole blood)[d]	15 mL	15 mL
TTS AESI form[e]	*Continuous*	
Concomitant therapies[f]		

a. Day 1 refers to first awareness of the event, which might be later than the date of onset. Every effort should be made to report as much information as possible about the event to the sponsor in a reasonable timeframe. The investigator should contact the sponsor for input on the feasibility of collecting blood samples, including the need for additional samples based on the nature of the event.

b. Day 29 is to be calculated relative to the actual day of onset of the event. If the event is not resolved on Day 29, subsequent follow up assessments can be performed at unscheduled visits as needed until resolution of the event. If the event is reported to the investigator more than 28 days after the onset of the event, the AESI Day 29 visit becomes redundant and does not need to be performed. However, it is expected that medical records from the event are obtained.

c. Suspected AESIs must be reported to the sponsor within 24 hours of awareness irrespective of seriousness (ie, serious and non serious AEs) or causality assessment (see Section 8.3.6).

d. Whole blood samples (~2mL) will be used for immediate measurement of a platelet count (as part of a complete blood count, if applicable) in a local laboratory or substitute for local laboratory, depending on local feasibility towards turnaround time of sample processing. Serum (4 mL) and plasma (2.5 mL) samples will be derived from the whole blood samples (8 mL and 5 mL, respectively) for coagulation related testing in a central laboratory (see Section 10.2, Appendix 2). For the follow up visit, the volume of blood to be collected may vary depending on the clinical evaluation of the case. All local laboratory results need to be encoded in the eCRF, including platelet counts. Low platelet counts are to be recorded as suspected AESIs (thrombocytopenia).

e. Medical information on local case management will be collected. Upon becoming aware of the suspected AESI, study site personnel should provide information on an ongoing basis. See Section 8.3.6 and Section 10.11, Appendix 11 for further details.

f. Refer to Section 8.3.6 for collection and recording of concomitant therapies associated with a suspected AESI.

AESI adverse event of special interest; CDC Centers for Disease Control and Prevention; d days(s); PF4 platelet factor 4; TTS thrombosis with thrombocytopenia syndrome

2. INTRODUCTION

Ad26.COV2.S (also known as Ad26COVS1) is a monovalent vaccine composed of a recombinant, replication-incompetent Ad26 vector, constructed to encode the S protein derived from a SARS-CoV-2 clinical isolate (Wuhan, 2019, whole genome sequence NC_045512), stabilized in its prefusion conformation.

On 27 February 2021, the US Food and Drug Administration (FDA) issued emergency use authorization (EUA) for Ad26.COV2.S for the prevention of COVID-19. On 11 March 2021, the European Commission granted conditional marketing authorization for the Ad26.COV2.S vaccine. Other regulatory agencies have subsequently authorized or (conditionally) approved the vaccine. A single dose of Ad26.COV2.S (5×10^{10} vp) has been granted EUA for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 18 years of age and older. The purpose of the current study is to evaluate safety and immunogenicity of 1 dose of Ad26.COV2.S in pregnant women.

For the most comprehensive nonclinical and clinical information regarding Ad26.COV2.S, refer to the latest version of the Investigator's Brochure (IB) for Ad26.COV2.S (IB 2021) and its addenda.

The term "study vaccine" throughout the protocol, refers to Ad26.COV2.S as defined in Section 6.1. The term "sponsor" used throughout this document refers to the entities listed in the Contact Information page(s), which will be provided as a separate document. The term "participant" throughout the protocol refers to the common term "subject."

COVID-19 Vaccine and Considerations

Currently, there is only limited data about the safety of vaccines for the prevention of COVID-19 in pregnant women. The development of a safe and effective COVID-19 vaccine is considered critical to contain the current outbreak and help prevent future outbreaks. COVID-19 vaccination is recommended for people who are trying to get pregnant now or might become pregnant in the future, as well as their partners (CDC 2021).

The quantitative correlate of protection against SARS-CoV-2 infection has not yet been identified in humans. Based on experience with severe acute respiratory syndrome coronavirus (SARS-CoV) and, more recently, in SARS-CoV-2 vaccine efficacy studies and mechanism of protection studies in nonhuman primates (NHP), binding and neutralizing antibody responses have been identified as the predominant correlates of protection (MacMahan 2020, Tostanoski 2020, Yu J 2020), and the correlation has been confirmed for Ad26.COV2.S in NHP and Syrian hamster challenge studies (Roozendaal 2021, Van der Lubbe 2021).

Adenoviral-vectored Vaccines

Recombinant, replication-incompetent adenoviral vectors are attractive candidates for expression of foreign genes for a number of reasons. The adenoviral genome is well characterized and comparatively easy to manipulate. Adenoviruses exhibit broad tropism, infecting a variety of

dividing and non-dividing cells. The adenoviral vaccine (AdVac®) vector platform, developed by Crucell Holland B.V. (now Janssen Vaccines & Prevention B.V.) allows for high-yield production of replication-incompetent adenovirus vectors, eg, Ad26, with desired inserts. The adenovirus E1 region is deleted to render the vector replication-incompetent and create space for transgenes, with viral replication taking place in cells that complement for the E1 deletion in the virus genome. Ad26 has been selected as a potential vaccine vector because there is substantial nonclinical and clinical experience with Ad26-based vaccines that demonstrate their capacity to elicit strong humoral and cellular immune responses and their acceptable safety profile, irrespective of the antigen transgene (see also Section 2.3.1).

Before the development of Ad26.COV2.S, the immunogenicity profile of Ad26-vectored vaccine candidates has been illustrated by data obtained following the immunization of adults with Ad26-vectored human immunodeficiency virus (HIV) vaccines (Ad26.ENVA.01, Ad26.Mos.HIV and Ad26.Mos4.HIV), an Ad26-vectored Ebola virus vaccine (Ad26.ZEBOV), Ad26-vectored respiratory syncytial virus (RSV) vaccines (Ad26.RSV.FA2 and Ad26.RSV.preF), an Ad26-vectored Zika virus vaccine (Ad26.ZIKV.001), and an Ad26-vectored malaria vaccine (Ad26.CS.01). Antigen-specific antibody responses are observed in almost all participants after 1 dose, in both naïve and pre-immune individuals (RSV). These antibodies may persist for a year or more (RSV) after a single vaccination in pre-immune participants. They have functional properties of neutralization (RSV, Zika), crystallizable fragment (Fc)-mediated antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (HIV, malaria). Furthermore, these data support an immunogenicity profile with emphasis on T-helper cell type 1 (Th)1 responses and demonstrate predominantly interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) production in cluster of differentiation (CD) 4^+ and $CD8^+$ T cells. (Barouch 2013, Milligan 2016, Mutua 2019, Salisch & Stephenson 2021).

Ad26.COV2.S Candidate Vaccine

The aim of the COVID-19 vaccine clinical development program is to develop a safe and effective vaccine for the prevention of COVID-19. The candidate vaccine to be assessed in this study is Ad26.COV2.S, which is a recombinant, replication-incompetent Ad26 encoding a prefusion stabilized variant of the SARS-CoV-2 S protein. The parental S protein sequence was derived from a SARS-CoV-2 clinical isolate (Wuhan, 2019, whole genome Sequence NC_045512). The selection of antigen was based on previous work on the SARS-CoV and MERS-CoV candidate vaccines (Chen 2005, Faber 2005, Modjarrad 2019). The S protein is the major surface protein on coronaviruses and is responsible for binding to the host cell receptor and mediating the fusion of host and viral membranes, thereby facilitating virus entry into the cell (Zumla 2016). In this study, the Ad26.COV2.S vaccine is administered and was assessed in 22 healthy adult participants in their 2^{nd} and/or 3^{rd} trimester of pregnancy at a single dose of 5×10^{10} vp (Sentinel and Safety Cohorts).

SARS-CoV-2 Virology and COVID-19 Disease Burden

SARS-CoV-2 is an enveloped, positive-sense, single-stranded ribonucleic acid (RNA) Betacoronavirus (CSG 2020, Wu 2020). It was first identified following reports of a cluster of acute respiratory illness cases in Wuhan, Hubei Province, China in December 2019 (Chen 2020,

Li 2020). Genomic sequencing performed on bronchoalveolar lavage fluid samples collected from patients with viral pneumonia, which identified a novel RNA virus from the family Coronaviridae (Lu 2020, WHO 2005). Phylogenetic analysis of the complete viral genome revealed that the virus, SARS-CoV-2, is part of the subgenus Sarbecovirus of the genus Betacoronavirus, and is most closely related (approximately 88% identity) to a group of SARS-CoV-like coronaviruses previously sampled from bats in China (Lu 2020, Zhou 2020).

SARS-CoV-2 has spread rapidly and globally since its emergence. The World Health Organization (WHO) declared that the outbreak constituted a public health emergency of international concern on January 30, 2020, and declared the outbreak to be a pandemic on March 11, 2020 (WHO 2005, WHO 2020b, WHO 2004). As of 18 March 2021, approximately 121,382,067 cases and approximately 2,683,209 COVID-19-related deaths have been reported worldwide. In the US, approximately 29,609,194 cases and 538,124 deaths have been reported (J Hopkins 2020). As of 18 March 2021, approximately 24,175,984 cases and 577,310 deaths have been reported in the European Union/European Economic Area (EU/EEA: ECDC 2020a) and the United Kingdom (ECDC 2020b).

Respiratory symptoms of COVID-19 typically appear 5 to 6 days following exposure to the virus, but may appear from 2 to 14 days following exposure, with the clinical manifestations ranging from mild symptoms to severe illness or death (CDC 2020a,c; Guan 2020; Linton 2020; UC San Diego; WHO 2020a). Descriptions of COVID-19 clinical case definitions compiled by the US Centers for Disease Control and Prevention (CDC) and Janssen-sponsored interviews with COVID-19-experienced clinicians include the following: signs and symptoms of respiratory distress such as blue lips, extreme shortness of breath and dyspnea, persistent cough, deep vein thrombosis (DVT), Kawasaki-like disease, discoloration of feet and toes, chills, shaking chills, loss of sense of taste and smell, signs of stroke, disorientation, inability to respond or understand verbal communication, among others. Other less common gastrointestinal symptoms have been reported by CDC (nausea, vomiting, diarrhea) (CDC 2020c).

Pregnant women may also be at increased risk for severe COVID-19 due to changes in their immune system and respiratory physiology. Furthermore, adverse birth outcomes, such as preterm delivery and stillbirth, may be more common among women infected with SARS-CoV-2 during pregnancy. Currently, information relating to the impact of SARS-CoV-2 infection on pregnancy, is limited. The US Centers for Disease Control (CDC) Immunization Safety Office, Vaccine Safety Datalink (VSD) found that over a period of 3 months (March 01-May 30, 2020), out of a total of 4,408 persons hospitalized with a COVID-19 diagnosis, 105 (2.4%) pregnant women with SARS-CoV-2 infection were hospitalized, including 62 (59%) women admitted for obstetric reasons (ie, labor and delivery or another pregnancy-related indication) of which 12 (19.4%) had COVID-19 comparable symptoms and 50 (80.6%) were asymptomatic; and 43 (41%) women were hospitalized for COVID-19 related symptoms without an obstetric reason. Of those 43 pregnant women that were hospitalized due to COVID-19 related symptoms, 13 (30%) required intensive care unit (ICU) admission, 6 (14%) required mechanical ventilation, and one patient admitted at 15 weeks gestation died from COVID-19. Furthermore, there was an increased prevalence of pre-pregnancy obesity (a body mass index ≥30 kg/m^2) and gestational diabetes in pregnant women

hospitalized for COVID-19 related symptoms as compared to pregnant women hospitalized for obstetric reasons. In pregnant women hospitalized with COVID-19, preterm delivery prevalence was 15.1% overall and 12.2% among live births, almost 70% higher than VSD baseline rates recorded (8.9% overall out of 43,571 live births and still births) during the study period. The presence of stillbirth (3.2%) was almost 4 times higher among women with SARS-CoV-2 than the VSD baseline rate during the study period of 0.6% (Panagiotakopoulos 2020).

A separate analysis of 11 case studies on perinatal outcomes of neonates born to women infected with SARS-CoV-2 during pregnancy, that was conducted in a cohort of 65 women and 67 neonates, found that the most common outcome of infection during pregnancy included fetal distress, reported in 30% of pregnancies, with 37% of women delivering preterm. Neonatal complications included respiratory distress pneumonia (18%), disseminated intravascular coagulation (3%), and asphyxia (2%) (Zimmermann 2020).

Clinical manifestations of disease are less severe in children than adults, with the majority of cases reported as being asymptomatic, mild or moderate (CDC 2020e, Deming 2006, Dong 2020). A study of 149,082 COVID-19 cases reported in the US found that only 1.7% of these cases occurred in persons aged <18 years, with relatively few pediatric COVID-19 cases hospitalized, indicating that COVID-19 might have a mild course among younger patients. Hospitalization was most common among pediatric patients aged <1 year and those with underlying conditions, with the highest proportion of severe and critical cases, by age group, reported for children aged <1 year of age (10.6% of cases in this age group), indicating that younger children, particularly infants, are more susceptible than older children to severe disease (CDC 2020 d,i).

Although the understanding of the epidemiology and clinical spectrum of COVID-19 is still evolving, the disease burden is rapidly mounting, highlighting the urgent medical need for a prophylactic vaccine.

It is not known if SARS-CoV-2 will remain as a worldwide pandemic. It is also not known if immunity is acquired after symptomatic or asymptomatic SARS-CoV-2 infection and how long it might last. Currently, the only preventive measures that have been employed with some success have been social distancing and quarantine after contact tracing and testing. Test and treat approaches await an effective proven safe therapy that can be implemented on a mass scale. It is generally believed that an effective vaccine will be one of the most important tools to help control this highly contagious respiratory virus.

The sponsor has developed a COVID-19 vaccine based on a human replication-incompetent Ad26 vector encoding the SARS-CoV-2 S protein. The S protein is the major surface protein of coronaviruses. Different animal models have been used for the evaluation of candidate coronavirus vaccines against SARS-CoV (2002-2003 outbreak), and the common conclusion that has emerged from the evaluation of several different vaccines is that the viral S protein is the only significant target for neutralizing antibodies (Buchholz 2004, Sui 2005, Zhang 2004, Zhou 2004) and the only viral protein that can elicit protective immunity in animal models (Berry 2004, Bisht 2004, Bukreyen 2004, Subbarao 2004, Yang 2004). Based on these findings, the S protein was selected as the sponsor's candidate vaccine antigen.

2.1. Study Rationale

Although data are still being accrued on the risks associated with developing COVID-19 during pregnancy, increased rates of complications, Caesarean sections, preterm delivery and of stillbirth have been observed during pregnancy with SARS-CoV-2 infection. In addition, it appears from current reviews that pre-pregnancy obesity and pregnancy diabetes are risk factors for severe COVID-19 in this population (Panagiotakopoulos 2020). Therefore, access to vaccination against COVID-19 is warranted during pregnancy. Furthermore, health care workers, who may be among the first groups targeted for vaccination, include people of reproductive age who may be pregnant, and therefore there is a need to understand the safety and reactogenicity of the Ad26.COV2.S vaccine in this population.

There is an increased risk of severe COVID-19 during pregnancy, as well as an increased risk of adverse birth outcomes (Villar 2021). Therefore, the aim of this study is to assess the safety, reactogenicity and immunogenicity of Ad26.COV2.S in adult participants in the 2nd and/or 3rd trimester of pregnancy. Due to the inherently higher rates of spontaneous abortions during the 1st trimester of pregnancy, participants will be vaccinated in the 2nd and/or 3rd trimester of pregnancy. A potential benefit of this approach is the higher levels of protective maternal antibodies that are expected to be transferred to the infant at birth in participants vaccinated during the 2nd and 3rd trimester (Munoz 2014). Phase 1 and 2 clinical studies VAC31518COV1001, COV1002, and COV2001 and the Phase 3 efficacy studies VAC31518COV3001 and COV3009 have demonstrated post-dose 1 safety of the vaccine in adults. The preclinical embryofetal and pre-and postnatal development toxicity (EFPPND) study (Study TOX14389) confirmed the absence of developmental and reproductive toxicity in animals. Please refer to the IB (IB 2021) and its addenda for VAC31518 (JNJ-78436735) for the most current information.

2.2. Background

Nonclinical Pharmacology

Nonclinical studies were performed to test the immunogenicity of different vaccine candidates, leading to the selection of the current vaccine for this development program.

Nonclinical pharmacology was evaluated in murine, rabbit, Syrian hamster and nonhuman NHP animal models for immunogenicity and included assessment of immunological parameters relevant to the theoretical risk of vaccine-associated enhanced respiratory disease (VAERD). A single immunization with Ad26.COV2.S (5×10^{10} vp) was shown to be immunogenic, induce S protein binding antibodies, SARS-CoV-2 neutralizing antibodies and a Th1 mediated immune response. Efficacy was demonstrated in the Syrian hamster model, in which immunization with Ad26.COV2.S resulted in a significantly lower infectious viral load in the lungs, as compared with the Ad26 control vector. Histological analysis of lung tissue at the end of the SARS-CoV-2 challenge study showed minimal pulmonary pathology in the lungs of Ad26.COV2.S immunized animals. There was no signs of VAERD after SARS-CoV2 inoculation of Ad26.COV2.S immunized NHP, even under conditions of suboptimal immunity. More details are provided in the IB (IB 2021) and its addenda.

Nonclinical Safety

Biodistribution

To assess distribution, persistence, and clearance of the Ad26 viral vector platform, intramuscular (IM) biodistribution studies have been conducted in rabbits using an Ad26-based HIV vaccine, Ad26.ENVA.01, and an Ad26-based RSV vaccine, Ad26.RSV.preF. In the available biodistribution studies, the Ad26 vector did not widely distribute following IM administration in rabbits. Ad26 vector deoxyribonucleic acid (DNA) was primarily detected at the site of injection, draining lymph nodes and (to a lesser extent) the spleen. Clearance of the Ad26 vector from the tissues was observed. Both Ad26 vectors showed a comparable biodistribution despite carrying different antigen transgenes. These data further indicate that the Ad26 vector does not replicate and/or persist in the tissues following IM injection. These platform data are considered sufficient to inform on the biodistribution profile of Ad26.COV2.S for which the same Ad26 vector backbone is used.

Toxicology

A combined GLP repeated dose toxicity and local tolerance study with Ad26.COV2.S was conducted in male and female New Zealand White (NZW) rabbits. In this study, Ad26.COV2.S was well tolerated when administered on 3 occasions over 4 weeks (ie, every 2 weeks) at 1×10^{11} vp/dose. The observed changes were related to a normal, anticipated (local and systemic) immunologic response to vaccination, and consisted clinically of (rare) transient local injection site dermal reactions, with transient minimal hyperthermia and minimal body weight loss or lower body weight gain after injection. This was associated with a transient (acute phase/immune) response in clinical pathology parameters, characterized by increases in plasma proteins (C-reactive protein, fibrinogen and globulins), and white blood cell counts (monocytes and lymphocytes). Microscopic pathology findings of minimal to slight inflammation and hemorrhage were observed at the injection sites, along with increased lymphoid cellularity of germinal centers in popliteal and iliac lymph nodes and the spleen, which is consistent with an immune response to the vaccine administration. Overall, the findings were considered non-adverse and were partially or completely reversible after a 3-week treatment-free period. All vaccinated animals developed an antibody response against SARS-CoV-2 S protein, confirming responsiveness of the rabbits to the vaccine.

Reproductive and Developmental Toxicology

A GLP compliant combined embryofetal and pre- and postnatal development study was conducted with Ad26.COV2.S in female NZW rabbits. In this study, female NZW rabbits were injected IM with a control solution (0.9% sodium chloride) or 1×10^{11} vp Ad26.COV2.S on Day 1 (ie, 7 days prior to mating), Gestation Day (GD) 6 and GD 20. The study included 2 subgroups, 1 group consisting of animals that were necropsied on GD 29 and had a uterine and fetal examination (external, visceral, and skeletal exams), and 1 group consisting of animals that were allowed to give birth and in which the survival and development of the kits were evaluated through Lactation Day 28. There was no evidence of impaired female fertility, and the vaccine did not show any harmful effect with regard to embryofetal or postnatal development. The parental females as well

as their fetuses and offspring exhibited SARS-CoV-2 S protein-specific antibody titers, indicating that maternal antibodies were transferred to the fetuses during gestation.

Clinical Studies

The safety and immunogenicity (post-dose 1) of Ad26.COV2.S, administered at several dose levels, has been demonstrated in healthy adults in the first-in-human study VAC31518COV1001 (IB 2021 and its addenda, Sadoff 2020).

At the time of protocol Amendment 4 writing, a robust clinical safety database is available consisting of all adult participants who received at least 1 dose of Ad26.COV2.S in the clinical studies VAC31518COV1001, VAC31518COV1002, VAC31518COV1003, VAC31518COV2001, VAC31518COV3001, and VAC31518COV3009.

The Phase 1/2a and Phase 2a clinical studies VAC31518COV1001, VAC31518COV1002 and VAC31518COV2001 have shown that a single dose of Ad26.COV2.S elicited a SARS-CoV-2 neutralizing antibody (wtVNA) and SARS-CoV-2 Spike-binding antibody (S-ELISA) response by Day 15 (14 days post-dose 1) and Day 29 (28 days post-dose 1) in adult participants ≥18 to ≤55 years and ≥65 years of age.

Consistent with data from ongoing Phase 1 and Phase 2 studies, the results of both Study VAC31518COV3001 and VAC31518COV3009 indicate that a single dose of Ad26.COV2.S at a dose level of 5×10^{10} vp was well tolerated and no vaccine-related safety signals were observed. In VAC31518COV3009, data from the primary/final analysis (end of double-blind phase) show that the reactogenicity and tolerability profile after a second dose of Ad26.COV2 was comparable to receiving the 1st dose. In addition, the data show that after administration of a second dose vaccine efficacy was increased. The available data indicate that an increase in immunogenicity after a second dose of Ad26.COV2.S correlates with increased vaccine efficacy. This was also shown for COVID-19 caused by variants of concern (eg, Alpha [B.1.1.7] and Mu [B.1.621]) for which enough cases were accrued to draw conclusions.

Overall, all safety data show that Ad26.COV2.S administered at a 1-dose or 2-dose level of 5×10^{10} vp has an acceptable safety and reactogenicity profile in participants ≥18 years of age.

Refer to the IB (IB 2021) and its addenda for a high level description of all ongoing studies with Ad26.COV2.S.

Clinical Safety Experience With Ad26-based Vaccines

As described above, replication-incompetent Ad26 is being used as a vector in the development of vaccine candidates against diseases such as malaria, RSV, HIV, Zika virus and filovirus, and has been used in the now licensed Ebola virus vaccine (Zabdeno / Ad26.ZEBOV).

As of 21 December 2020, Ad26-based vaccines developed by the sponsor have been administered to approximately 193,000 participants. The majority of these participants (more than 153,000) are enrolled in an ongoing Ebola vaccine study in the Democratic Republic of the Congo and an ongoing immunization campaign in Rwanda (UMURINZI Ebola Vaccine Program campaign) and

approximately 35,000 participants are enrolled in other ongoing studies. The remainder of participants were included in studies that have been completed.

The sponsor's clinical adenoviral vaccine (vector platform) (AdVac®) safety database report (V5.0, dated 10 April 2020, cut-off date 20 December 2019) describes integrated safety data from 26 completed clinical studies using Ad26-based vaccines for which the database was locked for final analysis. In these 26 studies, 4,224 adult participants were vaccinated with an Ad26-based vaccine and 938 adult participants received a placebo. A total of 6,004 Ad26-based vaccine doses were administered to adults. Most adult participants (3,557 of 4,224; 84.2%) received Ad26-based vaccine at a dose level of 5×10^{10} vp, while 284 adult participants (6.7%) received Ad26-based vaccine at the 1×10^{11} vp dose level (the highest dose level tested).

Overall, the Ad26-based vaccines were well tolerated, irrespective of the antigen transgene, without significant safety issues identified to date. See Section 2.3.1 for a summary of data from the AdVac® safety database report.

Ad26-based Vaccines in Pregnant Women

A review of individual case safety reports of pregnancy exposures performed by the Sponsor for all ongoing and completed Ad26-based vaccine clinical studies (covering a range of dose levels from 1×10^{9} vp to 1.6×10^{11} vp), identified 22 studies in which pregnancies were reported up to a cut-off date of 31 December 2020. These included clinical trials for severe acute respiratory syndrome coronavirus 2 (COVID-19) vaccine, Ebola vaccine, HIV vaccine, Human Papilloma Virus (HPV) vaccine, RSV vaccine and Zika vaccine.

The reports included a total of 1,618 pregnancies, of which 1,615 female study participants reported exposure to an Ad26 vectored vaccine product or control. Of these 1,615 cases, 1,421 reported potential exposure to the vaccine product during pregnancy whereas the remaining 194 cases were excluded as these cases reported either vaccine exposure outside pregnancy window or no exposure during pregnancy. The 1,421 cases corresponded to 1,408 individual pregnancies (1,395 mother cases, 2 mother/father linked pairs, and 11 mother/child pairs). Of these 1,408 pregnancies, 1,406 pregnancies were reported in female study participants whereas the remaining 2 pregnancies were reported in female partners of the male study participants, both of which reported exposure to Ad26.ZEBOV vaccine. The majority of these pregnancy cases (1,061 pregnancy cases reported out of a total of 20,432 participants enrolled) originated from the ongoing immunization campaign in the Democratic Republic of the Congo from the Ebola vaccine program, where pregnancy is not an exclusion criterion.

Overall, review of the available data obtained from over 1,600 reported pregnancies, with over 900 reported pregnancy outcomes in clinical trials with Ad26-based vaccines, is not suggestive of a pregnancy-related safety concern. There is no evidence of an increased risk of adverse outcomes either in the mother or the infant when administered within 3 months prior to onset of pregnancy or during pregnancy.

Th1/Th2 Profile of Ad26-based Vaccines in Clinical Studies

In the 1960s, a formalin-inactivated (FI) RSV vaccine was associated with enhanced respiratory disease (ERD) in young children, characterized by an increased rate of RSV-mediated, severe lower respiratory tract infection in the vaccinated individuals compared with the control group (Chin 1969, Fulginiti 1969, Kapikian 1969, Kim 1969). Although the mechanisms for ERD are not fully understood, it is thought that the FIRSV -vaccine may have: 1) failed to induce adequate neutralizing antibody titers; 2) led to an overproduction of binding antibodies promoting immune complex deposition and hypersensitivity reactions; 3) failed to induce adequate numbers of memory cluster of differentiation (CD)8^+ T cells important for viral clearance; and 4) induced a T helper cell (Th) type 2-skewed type T-cell response (Moghaddam 2006). Vaccine-induced ERD has also been described for SARS-CoV and MERS-CoV in some animal models in which candidate vaccines induced a Th2 -biased immune response (Agrawal 2016, Bolles 2011, Deming 2006, Honda-Okubo 2015, Houser 2017, Smatti 2018), but proof of human SARS-CoV or MERS-CoV vaccine-associated enhanced disease does not exist as these candidate vaccines were never tested for efficacy nor used in outbreak situations. For SARS and MERS, the mechanism of enhanced disease observed in mice has been associated with a Th2-mediated eosinophilic infiltration in the lung, which is reminiscent of ERD effects observed after RSV infection of mice immunized with FI-RSV. Similar to RSV vaccines, enhanced disease has been shown for whole-inactivated SARS-CoV vaccines, as well as subunit vaccines inducing a Th2-type immune response, which can be rescued by formulating vaccines in Th1-skewing adjuvants. In addition to a Th1-biased immune response, also induction of a high proportion of neutralizing antibodies compared with virus binding antibodies is desirable to prevent predisposition to enhanced disease as observed for RSV vaccines. While vaccine--associated enhanced disease was observed in nonclinical studies with experimental SARS and MERS vaccines, it is not a given that the same risk applies to COVID-19 vaccines. To the sponsor's knowledge, antibody-related COVID-19 disease enhancement has not been observed in nonclinical models yet. Antibodies against the receptor binding domain of SARS-CoV-2 were shown not to enhance in vitro infectivity. Repeated SARS-CoV-2 challenge of NHP or NHP studies with Th2-biasing COVID-19 vaccines that would be expected to predispose to enhanced disease did not show any signs of enhanced disease. In addition, disease enhancement was not observed in NHP immunized with ChAdOx1 encoding SARS-CoV-2 S protein prior to challenge with SARS-CoV-2 (IB 2021 and its addenda). The Ad26 vector was chosen due to its ability to induce humoral and strong cellular responses with a Th1 immune phenotype (Anywaine 2019, Barouch 2018, Colby 2020, Milligan 2016, Salisch 2019, Stephenson 2020, van der Fits 2020, Widjojoatmodjo 2015, Zahn 2012). This type 1 polarity of the immune response minimizes the risk of enhanced disease after SARS-CoV-2 infection.

The immunogenicity profile of adenoviral vectors, with particular emphasis on Th1 responses, is illustrated by data obtained from immunization of adults with Ad26-vectored HIV vaccines (Ad26.ENVA.01 and Ad26.Mos.HIV) and Ad26-vectored Ebola vaccine (Ad26.ZEBOV). These data show predominantly IFNγ and TNFα production in $CD4^+$ and $CD8^+$ T cells (Anywaine 2019, Barouch 2013, Barouch 2018).

The initial clinical immunogenicity data from Study VAC31518COV1001 shows that the Ad26.COV2.S vaccine induces robust neutralizing and binding antibody responses, a Th1-skewed phenotype, and robust CD4+ and CD8+ T-cell responses. Furthermore, results from the safety and reactogenicity analyses showed that the 5×10^{10} vp and 1×10^{11} vp Ad26.COV2.S dose levels had an acceptable tolerability in participants aged ≥18 to ≤55 years and in participants aged ≥65 years of age, with no significant safety issues in either age group during the observation period. The induction of a Th1-skewed response in all participants in both Ad26.COV2.S vaccine dose groups, indicates that the theoretical risk for predisposition of VAERD is minimal (IB 2021 and its addenda).

2.3. Benefit-Risk Assessment

More detailed information about the known and expected benefits and risks of Ad26.COV2.S may be found in the IB (IB 2021) and its addenda.

2.3.1. Risks Related to Study Participation

The following potential risks for Ad26.COV2.S will be monitored during the study and are specified in the protocol:

Risks Related to Ad26.COV2.S

Overall, all safety data (including reactogenicity) to date, show that Ad26.COV2.S at a dose level of 5×10^{10} vp as a 1-dose or 2-dose schedule, has an acceptable safety and reactogenicity profile in participants ≥18 years of age. Reactogenicity to Ad26.COV2.S in adults ≥18 years of age was demonstrated to be transient and most solicited AEs generally resolved in 1 to 2 days post-vaccination.

The most frequently reported solicited (local and systemic) AEs (collected up to 7 days after vaccination in the safety subset population) were vaccination site pain, fatigue, headache, and myalgia. Most AEs were of mild or moderate severity, were transient in nature and generally resolved within 1 to 2 days post-vaccination. Pyrexia was reported in 9.0% of participants receiving a single dose of Ad26.COV2.S at the 5×10^{10} dose level, as compared to 0.6% of participants in the placebo group. Grade 3 pyrexia was reported in 0.2% of participants in the Ad26.COV2.S group, of which the majority occurred in the younger age group. No Grade 3 pyrexia was reported in the placebo group. All fevers started on the day of vaccination or the day after and had a median duration of 1 day after vaccination with Ad26.COV2.S. Antipyretics were recommended post-vaccination for symptom relief as needed and were used more frequently in the Ad26.COV2.S group compared to placebo. Otherwise, the reactogenicity profile of Ad26.COV2.S was generally similar across sex, race/ethnicities, geographies, comorbidities, SARS-CoV-2 or HIV serostatus at baseline. The most frequently reported unsolicited AEs (collected up to 28 days after vaccination in the safety subset population) were fatigue, myalgia, and headache. Most were of mild or moderate severity, and most were considered not related to the study vaccine by the investigator.

For the most comprehensive nonclinical and clinical information regarding Ad26.COV2.S, refer to the latest version of the IB (IB 2021) and its addenda.

Sites should advise participants that side effects include fever as well as injection site pain, headache, fatigue, myalgia, and nausea per the current ICF. Anaphylaxis has been identified as a risk for Ad26.COV2.S. Individuals should be observed by a healthcare provider after vaccination per protocol requirements and in accordance with local regulations. Refer to the latest version of the IB (IB 2021) and its addenda (if applicable) for further details.

Thrombosis in combination with thrombocytopenia (TTS), in some cases accompanied by bleeding, has been observed very rarely following vaccination with Ad26.COV2.S. These cases occurred approximately 3 weeks following vaccination, mostly in women under 60 years of age. Thrombosis in combination with thrombocytopenia can be fatal. The exact physiology of TTS is unclear. Post-vaccination TTS is considered an identified risk for Ad26.COV2.S.

Participants should be instructed to seek immediate medical attention if they develop symptoms such as shortness of breath, chest pain, leg swelling, persistent abdominal pain, severe or persistent headaches, blurred vision, skin bruising and/or petechiae beyond the site of vaccination. The medical management of thrombosis with thrombocytopenia is different from the management of isolated thromboembolic diseases. Study site personnel and/or treating physicians should follow available guidelines for treatment of thrombotic thrombocytopenia (eg, from the American Society of Hematology 2021, British Society of Haematology - Expert Haematology Panel 2021, and the CDC 2021). The use of heparin may be harmful and alternative treatments may be needed. Consultation with a hematologist is strongly recommended. Management of the participant should not be delayed by decision making of the AESI Adjudication Committee.

Due to the possibility of the occurrence of TTS after vaccination with Ad26.COV2.S, additional reporting and data collection procedures have been included in the study for thrombotic events, thrombocytopenia, and TTS (see Section 8.3.6.1), which may facilitate diagnosis and clinical management of the event.

Guillain-Barré syndrome (GBS) has been reported very rarely following vaccination with Ad26.COV2.S. As a result, it is considered an identified risk. Investigators should be alert to GBS signs and symptoms to facilitate diagnosis, and to initiate adequate supportive care and treatment.

For the most comprehensive nonclinical and clinical information regarding Ad26.COV2.S, refer to the most recent version of the IB (IB 2021) and its addenda.

Risks Related to Ad26.COV2.S Administration after Previous Vaccination Ad26.COV2.S

Preliminary safety data of an Ad26.COV2.S booster (5×10^{10} vp, 2.5×10^{10} vp, or 1×10^{10} vp) administered ≥6 months post-primary single dose Ad26.COV2.S (5×10^{10} vp) vaccination are available from 244 participants (dose level blinded data). The data indicate that the safety and reactogenicity of a second Ad26.COV2.S dose is acceptable and in line with the safety and reactogenicity observed after the Ad26.COV2.S dose. There is no indication of increased

reactogenicity upon administration of a second dose of Ad26.COV2.S and no safety concerns have been observed.

In addition, the primary analysis of the study COV3009 indicated that the safety profile of the Ad26.COV2.S vaccine remained consistent and was generally well tolerated when administered as a second dose according to the study schedule (vaccinations at Day 1 and Day 57) (Johnson & Johnson 2021)

Risks Related to Adenoviral-vectored Vaccines

The clinical AdVac® safety database (report version 5.0, dated 10 April 2020, cut-off date 20 December 2019) contains pooled safety data from 26 Janssen-sponsored clinical studies with Ad26 vaccine candidates: Ad26.ZEBOV (Ebola; 10 studies), Ad26.ENVA.01, Ad26.Mos.HIV and Ad26.Mos4.HIV (HIV; 8 studies), Ad26.CS.01 (malaria; 1 study), Ad26.RSV.FA2 and Ad26.RSV.preF (RSV; 6 studies), and Ad26.Filo (filovirus; 1 study). In these studies, 4,224 adult participants and 650 children received at least 1 vaccination with an Ad26-based vaccine. The AdVac® safety database report includes data only from studies for which the database has been locked for the final analysis; therefore, of the studies including an Ad26.RSV.preF-based regimen mentioned in Section 2.2, only data for approximately 230 participants aged ≥60 years from studies VAC18193RSV1003, VAC18193RSV1005, and VAC18193RSV2003 were included.

Overall, the Ad26-based vaccines were well tolerated, without significant safety issues identified.

The majority of solicited local and systemic AEs were of mild or moderate severity and usually started within 1 to 2 days after vaccination. Most of the events resolved within 1 to 3 days.

In adults, the most frequently reported solicited local AE was injection site pain (56.9% of Ad26 participants, compared with 22.5% of placebo participants). All other solicited local AEs were experienced by less than 25% of adult participants. The most frequently experienced solicited local AE in children was injection site pain, reported in 13.9% of children aged 1-3 years, 29.8% of children aged 4-11 years, and 24.8% of children aged 12-17 years after vaccination with an Ad26-based vaccine. For placebo, these percentages were 29.2% in children aged 4-11 years and 14.3% in children aged 12-17 years. No children aged 1-3 years have received placebo.

Severe injection site pain was experienced by 1.0% of adult Ad26 participants and 0.8% of children aged 4-11 years. No children in the other 2 age groups and no placebo participants experienced severe injection site pain.

There was a trend toward an increase in the frequency of some local AEs with an increase in Ad26 dose, ie, injection site pain (18.7% of participants at the 0.8×10^{10} vp dose level, 38.7% of participants at the 2×10^{10} vp dose level, 52.0% of participants at the 5×10^{10} vp dose level, and 77.1% of participants at the 1×10^{11} vp dose level), and to a lesser extent injection site swelling (6.7%, 2.7%, 9.3%, and 17.6%, respectively). Injection site warmth was not collected at the 0.8×10^{10} vp and the 2×10^{10} vp dose level. The frequency of injection site warmth at the 5×10^{10} vp and the 1×10^{11} vp dose level was 19.5%, and 26.7%, respectively. This trend needs to be interpreted with caution since the participants in the lower dose groups (0.8×10^{10} vp and 2×10^{10} vp

dose level) were all from a single study (VAC52150EBL3002), and the majority of the participants in the highest dose group (1×10^{11} vp dose level) were also from a single study (VAC18193RSV2003).

The most frequently reported solicited systemic AEs (ie, reported in more than 30% of participants) for adult Ad26 participants were malaise (53.8%), fatigue (48.3%), headache (45.7%), and myalgia (38.3%), all of which were more frequent for Ad26 participants compared with placebo (36.4%, 30.7%, 30.0%, and 17.7% of placebo participants, respectively). Most of these events were considered related to the study vaccine. Pyrexia (9.9%) and vaccine-related pyrexia (9.0%) were also reported more frequently after administration of an Ad26-based vaccine compared with placebo (3.5% and 2.9%, respectively).

Solicited systemic AEs reported in ≥10% of children aged 1-3 years were decreased appetite (13.9%), decreased activity (13.2%), pyrexia (11.1%), and irritability (10.4%). The most frequently reported solicited systemic AEs in children aged 4-11 years (reported in ≥15% of Ad26 participants) were headache (23.6%; no data are available for the placebo arm in this age group), and decreased activity (18.5%) and irritability (17.6%), which were both reported in 4.2% (N 1) of placebo participants. The most frequently reported solicited systemic AEs in children aged 12-17 years (reported in ≥15% of Ad26 participants) were headache (34.6%) and fatigue (24.0%), compared to 33.3% and 19.0% of placebo participants, respectively. Most of the frequently experienced solicited systemic AEs in children were considered related to the study vaccine.

The majority of solicited systemic AEs were of mild or moderate severity. For adults, 6.5% of Ad26 participants and 2.0% of placebo participants reported severe solicited systemic AEs, mostly malaise and fatigue. Other severe solicited systemic AEs were reported in less than 3% of adult Ad26 participants.

There was a trend toward an increase in the frequency of solicited systemic AEs with an increase in Ad26 dose (35.3% at the 0.8×10^{10} vp dose level, 49.3% at the 2×10^{10} vp dose level, 64.5% at the 5×10^{10} vp dose level, and 70.4% at the 1×10^{11} vp dose level). The frequency of severe solicited systemic AEs also tended to increase with higher Ad26 dose, ie, 1.3% of participants at the 0.8×10^{10} vp and the 2×10^{10} vp dose level, 5.3% of participants at the 5×10^{10} vp dose level, and 14.4% of participants at the 1×10^{11} vp dose level. This trend needs to be interpreted with caution since the participants in the lower dose groups (0.8×10^{10} vp and 2×10^{10} vp dose level) were all from a single study (VAC52150EBL3002), and the majority of the participants in the highest dose group (1×10^{11} vp dose level) were also from a single study (VAC18193RSV2003).

The most frequently reported unsolicited AE in adult Ad26 participants was upper respiratory tract infection (5.3% vs 7.0% in adult placebo participants). The most frequently reported unsolicited AEs considered related to the vaccine were neutropenia (1.0% of adult Ad26 participants vs 0.5% of adult placebo participants) and dizziness (0.7% vs 0.2%, respectively).

General Risks Related to Vaccination

In general, IM injection may cause local itching, warmth, pain, tenderness, erythema/redness, induration, swelling, arm discomfort, or bruising of the skin. Participants may exhibit general signs

and symptoms associated with IM injection of a vaccine and/or placebo, including fever, chills, rash, myalgia, nausea/vomiting, headache, dizziness, arthralgia, general itching, and fatigue. These side effects will be monitored but are generally short-term. Instructions regarding use of antipyretic medication can be found in Section 6.8.

Syncope can occur in association with administration of injectable vaccines. Syncope can be accompanied by falls. Procedures should be in place to avoid falling injury. If syncope develops, participants should be observed until the symptoms resolve. Fear of injection might lead to fainting and fast breathing.

Participants may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, urticaria, or even anaphylaxis (see above risks related to Ad26.COV2.S). Severe reactions are rare. Participants with a known or suspected allergy, or history of anaphylaxis, or other serious adverse reactions to vaccines or their excipients (including specifically the excipients of the study vaccine) will be excluded from the study.

After vaccination, participants will remain at the study site for at least 1 hour (Sentinel and Safety Cohorts) and 30 minutes (the remaining participants) to be observed by the study staff. Necessary emergency equipment and medications must be available in the clinic to treat severe allergic reactions.

Pregnancy

The effect of the study vaccine on a fetus or on a nursing baby is unknown and will be evaluated in this study. See also Section 2.2.

Risks from Blood Draws

Blood draws may cause pain, tenderness, bruising, bleeding, dizziness, vasovagal response, syncope, and rarely, infection at the site where the blood is taken.

Risks from Collection of Nasal Swabs

Collection of a nasal swab may cause a nosebleed.

Risks from Genomic Research

Generally genomic research have little physical risk. Diagnostic testing will not be performed. The results of the study are intended for exploratory research purposes only (eg, RNAseq) and will not be provided to the participant.

Theoretical Risk of Enhanced Disease

Vaccine-associated enhanced disease has been described for SARS-CoV and MERS-CoV in some animal models (Agrawal 2016, Bolles 2011, Deming 2006, Honda-Okubo 2015, Houser 2017), and is associated with non-neutralizing antibodies and a Th2-skewed immune response, but proof of human SARS-CoV or MERS-CoV VAERD does not exist as these candidate vaccines were never tested for efficacy nor used in outbreak situations. In contrast, the Ad26-based vaccines have been shown to induce a clear Th1-skewed immune response and generate potent neutralizing

antibody responses in both humans and animal models (see Section 2.2). The initial clinical immunogenicity data from Study VAC31518COV1001 have also demonstrated the induction of a Th1-skewed response in all participants in both Ad26.COV2.S vaccine dose groups, indicating that the theoretical risk for predisposition of VAERD is minimal, also in case antibodies are transferred to the infant via the placenta (IB 2021 and its addenda). Participants in the present study will be informed of the theoretical risk of disease enhancement in the ICF. Furthermore, as a risk mitigation strategy, all participants in the study will be passively and actively monitored for acquisition of molecularly confirmed COVID-19 (see Section 4.1 and Section 8.1.2).

Unknown Risks

There may be other risks that are not known. If any significant new risks are identified, the investigators and participants will be informed

2.3.2. Benefits of Study Participation

Participants may benefit from clinical testing and physical examination.

Currently, there is only limited data about the safety of vaccines for the prevention of COVID-19 in pregnant women (refer to Section 2 for more details on pregnancy). The overall benefit and risk balance for individual participants thus, cannot be ascertained. Animal studies with COVID-19 Vaccine Janssen do not indicate direct or indirect harmful effects with respect to pregnancy, embryo/fetal development, parturition, or postnatal development.

The decision on whether to use the vaccine in pregnant women should be made in close consultation with a healthcare professional after considering the benefits and risks.

Cumulative review of pregnancies in clinical studies conducted by the Sponsor using Ad26 based vaccine constructs (based on data obtained from over 1,600 reported pregnancies, with over 900 reported pregnancy outcomes) did not reveal any safety concern related to Ad26-based vaccine exposure during pregnancy. Therefore, administration of Ad26.COV2.S in pregnancy may be considered when the potential benefits outweigh any potential risks to the mother and fetus.

Preliminary immunogenicity and safety data for an Ad26.COV2.S booster dose (5×10^{10} vp) at ≥6 months post-primary single dose Ad26.COV2.S administration, and efficacy data for a second dose of Ad26.COV2.S 2-3 months post-primary single dose Ad26.COV2.S administration support a favorable benefit-risk profile of booster administration in participants who received a single dose of Ad26.COV2.S.

2.3.3. Benefit-Risk Assessment for Study Participation

Based on the available data and proposed safety measures, the overall benefit-risk assessment for this clinical study is considered acceptable for the following reasons:

- Only participants who meet all inclusion criteria and none of the exclusion criteria (specified in Section 5) will be allowed to participate in this study. The selection criteria include adequate provisions to minimize the risk and protect the well-being of participants in the study.

- Safety will be closely monitored throughout the study:

  In general, safety evaluations will be performed at scheduled visits during the study, as indicated in the Schedule of Activities.

  After vaccination, participants will remain at the study site for at least 30 minutes (with participants in the Sentinel and Safety Cohorts under observation for at least 60 minutes) and will be monitored by study staff. Necessary emergency equipment and medications must be available in the clinic to treat severe allergic reactions. Adult participants will use a reactogenicity diary in the eCOA to document solicited signs and symptoms. Details are provided in Section 8.2 and Section 8.3.

  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.

  The investigator or the designee will document unsolicited AEs, serious adverse events (SAEs), AESI, pregnancy-related AEs throughout pregnancy, and MAAEs, as indicated in Section 8.2, Section 8.3, and Section 10.4.

  From the time of local approval of protocol Amendment 3 onwards, TTS is considered to be an AESI (Section 8.3.6). Suspected AESIs (thrombotic events and thrombocytopenia [defined as platelet count below 150,000/µL post-vaccination, see American Society of Hematology (ASH) 2021]) must be reported to the sponsor within 24 hours of awareness. Suspected AESIs will be followed up as described in the Schedule of Activities in Section 1.3.6.

  Any clinically significant abnormalities (including those persisting at the end of the study/early withdrawal) will be followed by the investigator until resolution or until clinically stable. For those abnormalities persisting at the end of the study/early withdrawal, the outcome should be documented in the participant's medical record.

- Several safety measures are included in this protocol to minimize the potential risk to participants, including the following:

  Eligibility criteria will be reassessed pre-vaccination on Day 1.

  There are prespecified rules for all participants, that if met would result in pausing of further vaccinations, preventing exposure of new participants to study vaccine until the IDMC reviews all safety data (see Section 6.9).

  All participants will be monitored in this study to diagnose COVID-19 infection, if applicable.

  Study vaccinations will be discontinued in participants for the reasons included in Section 7.

  Criteria to delay administration of the study vaccine are included in Section 5.5.

3. 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. • SAEs and AESI throughout the study (from vaccination until end of the study, ie, at least 12 months after delivery). • 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 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, ie, at least 12 months after delivery). • 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 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 (ie, 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 (ie, 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 (ie, in cord blood).	• Serological response to vaccination as measured by VNA titers at birth (ie, in cord blood).
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 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 PBMC and intracellular cytokine staining (ICS) including CD markers: CD4+/CD8+, tumor necrosis alpha (TNFα), interferon gamma (IFNγ), interleukin (IL)-2, IL-4, IL-5, IL-13, and/or other Th1/Th2 markers. OR • Dual or single IFNγ and IL-4 enzyme-linked immunospot (ELISpot) assay after stimulation of PBMCs with SARS-CoV-2 protein peptides.
To assess the impact of pre-existing humoral immunity against coronavirus other than	Analysis of antibodies binding to coronaviruses other than SARS-CoV-2, or

SARS-CoV-2 at baseline on Ad26.COV2.S vaccine immunogenicity.	other respiratory viruses by ELISA or equivalent assay.
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.
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. • 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 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 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.
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	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

timepoints, including after booster vaccination, if feasible.	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.
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. • 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 Ad26.COV2.S during the study period, up to delivery.	• 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.
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. • 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.
To further explore the impact of vaccination with Ad26.COV2.S on coagulation-related parameters: • In the event of a suspected AESI of 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 CBC with differential, including platelet counts (performed locally at the site: see Appendix 2, Table 6). • 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 Appendix 2, Table 7).
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.
To explore changes in the SARS-CoV-2 genome	• Identification of SARS-CoV-2 variants by sequencing of nasal swabs samples (as available)
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. • 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.
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.
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. • SARS-CoV-2 neutralizing titers in serum measured by VNA. • SARS-CoV-2-binding antibodies measured by ELISA: Analysis of antibodies binding to the SARS-CoV-2 S and/or N-protein.
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 VNA titers at birth (ie, 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. • 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 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 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.
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)

Refer to Section 8 for evaluations related to endpoints.

HYPOTHESIS

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.

The study will provide descriptive information regarding the safety and immunogenicity of Ad26.COV2.S administered as a booster dose in participants who had previously received a COVID-19 vaccine and in those that were vaccine naïve at study entry and receive a booster dose during the study.

4. STUDY DESIGN

4.1. Overall 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 diagram of the study design is provided in Section 1.2.

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 in this study. Efforts were made to ensure good representation in terms of race and ethnicity. The sample size of each of the groups were preferably equally distributed but flexible, and the target was to recruit at least 40 participants in Groups 1 to 3 and at least 25 participants in Group 4. As of Protocol Amendment 7 approval, participant enrollment in this study will be ceased. These participants will continue to be followed in this study in accordance with the protocol.

The first 22 vaccine naïve participants participated in the Sentinel and Safety Cohorts to assess safety and reactogenicity in the 1-dose regimen.

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) 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

These participants will receive 1 dose of Ad26.COV2.S at 5×10^{10} vp. Participants will be 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.

Enrollment was staggered and started with recruitment of vaccine naïve Sentinel participants followed by a larger Safety Cohort (n 17 participant, excluding sentinels) who all received 1 dose of Ad26.COV2.S vaccine at 5×10^{10} vp.

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

IDMC Review Outcome

Safety Profile Acceptable After IDMC Review

At the time of writing Amendment 5, 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 following review of the Sentinel and Safety Cohorts safety data. The remaining participants will receive 1 dose of Ad26.COV2.S at 5×10^{10} vp. The vaccination schedule is presented in Figure 2.

Booster Vaccinations

Previously Vaccinated Participants

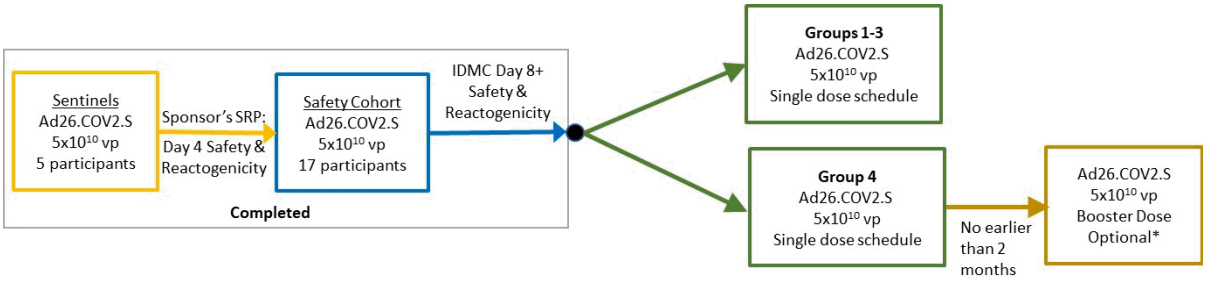
Previously vaccinated participants (Groups 1-3) will receive a single (booster) dose of Ad26.COV2.S at 5×10^{10} vp as part of the study. No further vaccinations by these groups will be received during the study.

Vaccine Naïve Participants

For participants that are vaccine naïve at study entry, a booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp will be offered to ongoing, consenting participants (Group 4, including Sentinel and Safety Cohorts) who have completed the pregnancy during which they were enrolled in the study, are not pregnant again, and have not received another COVID-19 vaccine (eg, national immunization program).

The booster vaccination will be administered not earlier than 2 months after completion of the participant's Ad26COV2.S vaccination in the study.

Figure 2: Decision Tree for Staggered Enrollment



A target of 240 adult participants (including 22 participants in the Sentinel and Safety Cohorts) in the 2nd or 3rd trimester of pregnancy (Week 16 to Week 38 of gestation, inclusive) was to be enrolled. As of Protocol Amendment 7 approval, participant enrollment in this study will be ceased.
Enrollment was staggered and started with recruitment of Sentinel participants followed by Safety Cohort (excluding Sentinels). At the time of writing Amendment 5, 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 following review of the Sentinel and Safety Cohorts safety data. The remaining participants were enrolled and randomized to receive 1 dose of Ad26.COV2.S at 5×10^{10} vp.

Previously vaccinated participants (Groups 1 to 3) should have received their last COVID 19 vaccination at least 4 months prior to receiving the study vaccine.

Participants will be 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.

* A booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp will be offered to ongoing, consenting participants (Group 4, including Sentinel and Safety Cohorts) who were vaccine naïve at study entry and who have completed the pregnancy during which they were enrolled in the study, are not pregnant again, and have not received another COVID 19 vaccine (eg, national immunization program). Please refer to Section 6.2 for further details.

IDMC Independent Data Monitoring Committee; vp virus particles

Study Duration

For each adult participant, the total study duration from screening until the last follow-up visit will be approximately 16 months. The study will consist of a 28-day screening phase, a study period

from vaccination to pregnancy completion/termination, and a follow-up period of 12 months post pregnancy completion/termination.

Neonates/infants born to the participants in the study will be followed for approximately 12 months postpartum (see Section 1.3.3).

The booster vaccination will be administered not earlier than 2 months after completion of the participant's Ad26.COV2.S vaccination in the study. The study duration will not be extended to accommodate a booster visit.

Study Procedures

Safety will be assessed by collection of solicited local (at injection site) and systemic AEs, unsolicited AEs, and SAEs. Other safety assessments include vital signs measurements (heart rate, supine systolic and diastolic blood pressure [adults only], respiratory rate, body temperature, etc.) and physical examinations at the time points indicated in the Schedules of Activities in Section 1.3.

After vaccination, adult participants will remain under observation at the study site, for at least 30 minutes (60 minutes for participants in the Sentinel and Safety Cohorts), for the presence of any severe acute reactions and solicited events.

Any solicited local or systemic AEs, unsolicited AEs, SAEs, AESI, pregnancy-related AEs throughout pregnancy, MAAEs, concomitant medications, and vital signs will be documented by study site personnel following this observation period. In addition, adult participants will record solicited signs and symptoms in a reactogenicity diary for 7 days post-vaccination.

The reporting periods of unsolicited AEs, SAEs, AESI, pregnancy-related AEs throughout pregnancy, MAAEs, and special reporting situations are detailed in Section 8.3. Reporting periods for concomitant therapy are outlined in Section 6.8.

In addition, participants will be asked daily if they have experienced any health concerns (including new onset of symptoms such as shortness of breath, chest pain, leg swelling, persistent abdominal pain, severe or persistent headaches or blurred vision, easy bruising, or tiny blood spots under the skin beyond the site of the injection) within the 30-day time period post-vaccination, and if so, participants will be advised to contact the study center. For Group 4 booster participants only, subjective changes in (postpartum) breast milk production (reduction) will be collected in the eCOA diary.

Adverse maternal/fetal outcomes and adverse neonate/infant outcomes SAEs (including MIS-C), MAAEs and AEs leading to discontinuation will be recorded. A final safety follow-up visit is scheduled 12 months postpartum for adult participants and approximately 12 months after birth for the neonates/infants.

From all adult participants and neonates/infants, blood samples will be collected at selected timepoints indicated in the Schedules of Activities in Section 1.3, for humoral immunogenicity assessments, with an emphasis on neutralizing and binding antibody responses. In addition, blood

samples will be collected at selected timepoints indicated in the Schedules of Activities in Section 1.3; analysis of vaccination-related biomarkers in PAXgene® tubes may be performed.

From a subset of approximately 25 adult participants per group, PBMCs were to be collected for analysis of cellular immunogenicity (including Th1/Th2 assessments). With only 1 participant in this subset at the time of writing this Protocol Amendment 7, PBMC sampling will be removed from the study as of Protocol Amendment 7 approval.

For vaccine naïve participants at study entry who receive a booster vaccination, blood samples will be collected from adults pre-boost and 28 days post-boost for humoral immunogenicity assessments and whole blood will be collected for the analysis of vaccination-related biomarkers.

Further details about the immunogenicity assessments are provided in Section 8.1.1.

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 eCOA.

For the duration of the study, each participant or parent(s)/caregiver(s) for the neonate/infant will be asked at least twice a week, through the eCOA, if the participant or their neonate/infant have experienced any new symptoms or health concerns that could be related to infection with SARS-CoV-2 ([suspected] COVID-19 surveillance [symptom check]).

All participants and neonates/infants with COVID-19-like signs or symptoms and all participants and neonates/infants with a positive RT-PCR test from outside the study meeting the prespecified criteria for suspected COVID-19 should undertake prespecified COVID-19 procedures as outlined in the Schedule of Activities in Section 1.3.4 and Section 1.3.5, respectively.

If neonates were born to mothers with confirmed COVID-19, or if a close relative from the same household as the neonate/infant has confirmed COVID-19, testing for SARS-CoV-2 should be performed for the neonate/infant, regardless of whether there are signs of infection in the neonate/infant. Details are provided in Section 8.1.2. The occurrence of asymptomatic SARS-CoV-2 infection will also be assessed, if feasible (see Section 8.1.4).

Site staff and participants will not be blinded as to the outcome of the molecular test results from the local (hospital) laboratory. Their routine HCP can obtain external diagnostics, including RT-PCR or other molecularly confirmed viral tests, as medically needed.

All necessary precautions (per local regulations) should be taken to protect site staff and other contacts of participants and neonates/infants who are confirmed to have COVID-19 until proven negative by molecular techniques or until resolution.

In the event of a confirmed SARS-CoV-2 infection, the participant or parent/caregiver (for neonate/infant) will be notified, and the participant or parent/caregiver (for neonate/infant) will be asked to adhere to the appropriate measures and restrictions as defined by local regulations.

Enrolled participants will be counselled on SARS-CoV-2 infection prevention each time that they have a contact with site staff, in line with local guidelines.

If a participant should have COVID-19 and their symptoms deteriorate, they will be instructed seek medical care.

An IDMC has been commissioned for this study. Refer to Committees Structure in Section 10.3.6

The planned primary and final analyses are detailed in Section 9.5 Planned Analyses.

Dose Level Selection

The rationale behind the selection of the dose level of Ad26.COV2.S is described in Section 4.2.

Study Population

Adult participants in the 2nd and/or 3rd trimester of pregnancy (ie, Week 16 to Week 38 of gestation, inclusive) will be recruited for the study.

Participants should either have completed a primary vaccine regimen of Comirnaty (Pfizer-BioNTech, Group 1), SpikeVax (Moderna, Group 1), Ad26.COV2-S (Janssen, Group 2) or from another manufacturer (Group 3) who have received their last COVID-19 vaccination at least 4 months prior to receiving the study vaccine; or be vaccine naïve (Group 4).

Participants with severe medical complications, obstetric risks or risk factors that put them at higher risk for complications will be excluded to avoid confounding of vaccine safety endpoints.

See also Sections 5.1 and 5.2.

Blinding, Control, Study Phase/Periods, Vaccine Groups

This is a non-randomized open-label study. There is no placebo group.

Biomarker Collection

For PAXgene® tubes collected in adult participants and in cord blood (if feasible), biomarker analysis may be performed to explore potentially informative biomarkers related to vaccine immunogenicity.

For adult participants with a positive test result for SARS-CoV-2 infection, biomarker analysis may be performed on in PAXgene® tubes 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.

4.1.1. Study-Specific Ethical Design Considerations

Potential participants will be fully informed of the risks and requirements of the study and, during the study, participants will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be

withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only participants who are fully able to understand the risks, benefits, and potential AEs of the study, and provide their consent voluntarily will be enrolled.

Adult participants in the 2nd and/or 3rd trimester of pregnancy and subsequently in their neonates/infants may not receive a tangible benefit from participation in the study, except for compensation for the time and inconveniences that may arise from participation in the study. See Section 2.3 for details on potential and known benefits and risks, and for the safety measures taken to minimize risk to participants.

The study-specific ethical considerations will be described in the ICF, where the terms legal guardian and legally acceptable representative refer to the legally appointed guardian of the neonate/infant with authority to authorize participation in research. For each neonate/infant, his or her parent(s) (both parents, if available) or legally acceptable representative(s), as required by local regulations, must give written consent (permission) according to local requirements after the nature of the study has been fully explained and before the performance of any study-related assessments.

For the purposes of this study, all references to neonate/infant participants who have provided consent refers to the neonate/infant participants and his or her parent(s) or the neonate/infant participant's legal guardian(s) or legally acceptable representative(s) who have provided consent according to this process.

The total blood volume to be collected from adults is considered to be an acceptable amount of blood to be collected over this time period from the population in this study based upon the US Department of Health and Human Services Office for Human Research Protections, and US (FDA) guidelines of 550 mL in any 8-week period (US HHS 1998, US HHS 2019), as well as the European Commission guidelines of 500 mL per donation and 3 L per consecutive 12 month period (98/463/EC).

The total blood volume to be collected from infants is considered to be acceptable according to guidelines (EU 2008, Howie 2011, Peplow 2019, US HHS 2019) and will not exceed 3.5 mL per blood draw for infants ≤6 months of age. Except cord blood, no blood will be collected from newborns.

4.2. Justification for the Regimen and Dose

The regimen and dose selection in this study are aimed primarily at providing information on safety, reactogenicity, and immunogenicity of Ad26.COV2.S at a dose level of 5×10^{10} vp in adult participants in the 2nd and/or 3rd trimester of pregnancy. All participants are planned to receive a single dose of Ad26COV2.S at 5×10^{10} vp.

The 5×10^{10} vp dose level is currently being evaluated in ongoing clinical studies VAC31518COV1001, VAC31518COV1002, VAC31518COV2001, VAC31518COV3001, and VAC31518COV3009. Interim analysis results from study VAC31518COV1001 have demonstrated safety and immunogenicity for the 5×10^{10} vp dose level, which has been confirmed in the other studies.

Overall, the reactogenicity data from the current study is consistent with the reactogenicity data observed in the Phase 1 study VAC31518COV1001 post-dose 1 and the Phase 2 study VAC31518COV2001 post-dose 1 for the groups with the same dose level (ie, 5×10^{10} vp dose level of Ad26.COV2.S).

Based on the VAC31518COV3001 primary analysis results, EUA for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 18 years of age and older was granted.

The most extensive clinical safety information is available from the Phase 3 study VAC31518COV3001 that has been conducted with ≥43,000 participants ≥18 years of age to date, and includes exposure to a single dose or 2-dose vaccination regimen with Ad26.COV2.S at the selected dose level (5×10^{10} vp) with at least a 56-day interval between doses for the 2-dose regimen. A total of 27,181 received at least 1 dose of Ad26.COV2.S at the selected dose level of 5×10^{10} vp. Overall, the results from the safety and reactogenicity analyses showed that the 5×10^{10} vp dose level of Ad26.COV2.S administered as a 1-dose regimen had an acceptable safety and reactogenicity profile with no significant safety issues identified. In general, a lower reactogenicity was observed for the older adults compared to the younger adults.

Based on the primary analysis data (cut-off date 22 January 2021), the results of this study indicate that a single dose of Ad26.COV2.S at a dose level of 5×10^{10} vp was well tolerated.

The safety of 2 doses of Ad26.COV2.S administered with a 56-day interval was evaluated in Study VAC31518COV3009. 16,751 participants received 2 doses of vaccine - 8,655 received Ad26.COV2.S and 8,096 received placebo. The data indicate that the safety and reactogenicity of a second Ad26.COV2.S dose is acceptable and in line with the safety and reactogenicity observed after the first Ad26.COV2.S dose. There is no indication of increased reactogenicity upon administration of a second dose of Ad26.COV2.S and based on the initial clinical assessment no safety concerns have been observed.

A single dose of Ad26.COV2.S vaccine is immunogenic and highly efficacious against severe COVID-19 disease and COVID-19 related hospitalization and death. Furthermore, while protection against variants of concern (such as the Beta and Mu variants in study COV3001 and the Delta variant in the Sisonke study [Gray 2021]) remains high against serious disease, hospitalization, and death, this protection is lower against, eg, the Gamma variant compared to the reference Wuhan strain.

Giving a second dose of Ad26.COV2.S results in marked increases of immune responses and those higher immune responses correlate with better protection against COVID-19, as shown in the primary analysis of study COV3009 (Johnson & Johnson 2021). The CDC (CDC 2021a) recently advised to give a booster vaccination, therefore, boosting will be offered to all eligible participants in this study who receive only a single vaccination with Ad26.COV2.S in the study. As the Janssen vaccine is approved as a single dose vaccine, participants who received 2 doses of Ad26COV2.S, if applicable, are considered to already have received the booster dose.

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

4.3. End of Study Definition

End of Study Definition

The end of study is considered as the last visit shown in the Schedule of Activities for the last participant in the study.

- For adult participants, the last visit is at approximately 12 months postpartum.
- For neonates/infants, the last visit is at approximately 12 months of age.

Study Completion Definition

An adult participant will be considered to have completed the study if she has completed assessments at the 12 months postpartum visit, or has experienced a clinical endpoint that precludes further continuation in the study.

An infant will be considered to have completed the study if he or she has completed assessments at the visit 12 months after birth or has experienced a clinical endpoint that precludes further continuation in the study.

All participants who prematurely discontinue study participation for any reason before completion of the 12 months postpartum visit will not be considered to have completed the study.

5. STUDY POPULATION

Screening for eligible participants will be performed within 28 days before study vaccination. Refer to Section 5.4 for conditions under which the repeat of any screening procedures are allowed.

The inclusion and exclusion criteria for enrolling participants in this study are described below. Eligibility criteria to receive the booster vaccination are described in Section 6.2.

If there is a question about these criteria, the investigator must consult with the sponsor to try to resolve any issues before enrolling a participant in the study. Waivers are not allowed.

5.1. Inclusion Criteria

Each potential adult participant must satisfy all of the following criteria to be enrolled in the study:

1. Participant must sign an ICF indicating that she understands the purpose, procedures and potential risks and benefits of the study, is willing to participate in the study. Within the ICF, the participant must also give consent to follow-up the neonate/infant after birth.
2. Criterion modified per Amendment 1

2.1 Participant is 18 (or the legal age of consent in the jurisdiction in which the study is taking place) to 45 years of age, inclusive, on the day of signing the ICF.

3. Criterion modified per Amendment 3

3.1 Participant must be healthy as confirmed by medical history, physical examination, vital signs, and obstetric history performed at Screening, and must not have medical history of comorbidities related to an increased risk of severe COVID-19 (refer to Exclusion Criterion 24) or high-risk pregnancy (refer to Exclusion Criterion 2). Participant may have underlying illnesses (eg, stable/well-controlled HIV infection*), as long as the symptoms and signs are medically controlled and not considered to be comorbidities related to an increased risk of severe COVID-19 or high-risk pregnancy.

* Stable/well-controlled HIV infection includes:

Criterion modified per Amendment 6

a. CD4 cell count ≥300 cells/μL

b. HIV viral load <50 copies/mL.

c. Participant must be on a stable anti-retroviral treatment (ART) for 6 months (unless the change is due to tolerability, in which case the regimen can be for only the previous 3 months; changes in formulation are allowed) and the participant must be willing to continue his/her ART throughout the study as directed by his/her local physician.

Note: Participants with ongoing and progressive comorbidities associated with HIV infection will be excluded but comorbidities associated with HIV infection that have been clinically stable for the past 6 months are not an exclusion criterion.

Laboratory methods for confirming a diagnosis of HIV infection are: Any evidence (historic or current) from medical records, such as ELISA with confirmation by Western Blot or PCR, or of a detectable viral load (country-specific regulatory approved tests). A laboratory result within 6 months of screening does not need to be repeated.

Criterion modified per Amendment 6

If a potential participant does not have HIV viral load and CD4 cell count data in their medical records from the last 6 months, efforts will be made to obtain the necessary data for potential entry into the study. A laboratory result within 6 months of screening does not need to be repeated.

4. Criterion modified per Amendment 4.

   a. If on medication for a condition, the medication dose must have been stable for at least 4 weeks preceding vaccination.

5. Participant will be included on the basis of physical examination, medical history, and vital signs[a].

6. Criterion modified per Amendment 1.

   a. Criterion modified per Amendment 4

[a] Participants may be enrolled with Grade 1 or Grade 2 toxicity gradings for vital signs measurements per the Investigator's discretion.

b. Participant will be at 2nd or 3rd trimester of pregnancy, ie, Week 16 to Week 38 of gestation (inclusive), at the time of vaccination, based on ultrasound at the time of screening (or not longer than 10 days prior to vaccination if performed elsewhere).

7. Participant agrees to not donate bone marrow, blood, and blood products from the first study vaccine administration until 3 months after receiving the last dose of study vaccine.
8. Participant must be willing to provide verifiable identification, has means to be contacted and to contact the investigator during the study.
9. Participant must have access to a consistent means of contact either by telephone contact or e-mail/computer.
10. Must be able to read, understand and complete questionnaires in a digital application (ie, The "COVID 19 signs and symptoms surveillance question," the "SIC" for adults and the "PedSIC" for children); and the reactogenicity diary (adults only).[a]
11. Criterion modified per Amendment 4.

    11.1 Criterion modified per Amendment 6

    a. Participant either received their last COVID-19 vaccination with an authorized/licensed COVID-19 vaccine (at least 4 months prior to first study vaccination) or is COVID-19 vaccine naïve.

5.2. Exclusion Criteria

Any potential participant who meets any of the following criteria will be excluded from participating in the study:

1. Criterion modified per Amendment 4

   1.1 Participants with medical or obstetric histories that put them at higher risk for maternal or fetal complications (eg, chronic pregnancy-related disorders, birth defects or genetic conditions during previous pregnancy).

2. Criterion modified per Amendment 4

   2.1 Participants with risk factors for the current pregnancy (eg, multiple pregnancies [twins or higher order multiples], unstable diabetes, high blood pressure, morbid obesity [before pregnancy], epilepsy, ongoing thyroid disease, heart or blood disorders, autoimmune disorders) or complications (eg, abnormal placenta position, fetal growth less than the 10th percentile for gestational age [fetal growth restriction]) for the current pregnancy.

3. Participants with close relatives with known congenital disorders or anomalies.
4. Participant with abnormal pregnancy screening test[b] (eg, ultrasound fetal abnormalities, maternal blood screen).
5. Criterion modified per Amendment 1.

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[a] Participants with visual impairment are eligible for study participation and may have assistance in completing the eCOA questionnaires

[b] https://www.cdc.gov/ncbddd/birthdefects/diagnosis.html, last reviewed on 05 Dec 2019.

5.1 Participant has a clinically significant acute illness (this does not include minor illnesses such as diarrhea or mild upper respiratory tract infection) or temperature ≥38.0ºC (100.4°F) within 24 hours prior to the planned first dose of study vaccine; vaccination at a later date (refer to Section 5.5) is permitted at the discretion of the investigator and after consultation with the sponsor.

6. Criterion modified per Amendment 4:

   6.1 Participant has a history of malignancy within 2 years before screening (exceptions are squamous, basal cell carcinomas of the skin, carcinoma in situ of the cervix, or a malignancy considered cured with minimal risk of recurrence).

7. Participant has a known or suspected allergy or history of anaphylaxis or other serious adverse reactions to vaccines or their excipients (including specifically the excipients of the study vaccine) (refer to the IB 2021 and its addenda).

8. Criterion modified per Amendment 4.

   8.1 Participant has abnormal function of the immune system resulting from:

   a. Clinical conditions (eg, potential immune mediated disease or known or suspected immunodeficiency, chronic kidney disease [with dialysis]) expected to have an impact on the immune response elicited by the study vaccine.

   Participants with clinical conditions stable under non-immunomodulator treatment (eg, autoimmune thyroiditis, autoimmune inflammatory rheumatic disease such as rheumatoid arthritis) may be enrolled at the discretion of the investigator.

   Note: Non-immunomodulatory treatment is allowed as well as steroids at a non-immunosuppressive dose or route of administration.

   Criterion modified per Amendment 6

   b.1 Chronic or recurrent use of systemic corticosteroids within 6 months before administration of study vaccine and during the study. A substantial immunosuppressive steroid dose is considered to be (>20 mg prednisone or equivalent daily for 2 consecutive weeks).

   Note: Ocular, topical or inhaled steroids are allowed.

   c. Administration of antineoplastic and immunomodulating agents or radiotherapy within 6 months before administration of study vaccine and during the study.

9. Criterion modified per Amendment 1

   9.1 Participant has a history of any serious, chronic, or progressive neurological disorders or seizures including GBS, with the exception of febrile seizures during childhood.

10. Participant has a history of chronic urticaria (recurrent hives), eczema or adult atopic dermatitis.

11. Criterion modified per Amendment 4.

    11.1 Participant received treatment with immunoglobulins in the 3 months or blood products in the 4 months before the planned administration of the study vaccine or has any plans to receive such treatment during the study.

    RHo (D) immunoglobulin injection can be given at any time.

12. Criterion modified per Amendment 1

    12.1 Participant received or plans to receive:

    a. Licensed live attenuated vaccines - within 28 days before or after planned administration of the 1st and booster study vaccinations

    b. Other licensed (not live) vaccines - within 14 days before or after planned administration of the 1st and booster study vaccinations.

13. Criterion modified per Amendment 4

    Criterion modified per Amendment 6

    13.2 Participant received an investigational drug or used an invasive investigational medical device within 30 days, or received investigational immunoglobulin or monoclonal antibodies within 3 months, or received convalescent serum for COVID-19 treatment within 4 months or received an investigational vaccine (including investigational Adenoviral-vectored vaccines and vaccines against COVID-19) within 4 months, before the planned administration of the first dose of study vaccine or is currently enrolled in another investigational study during the course of this study.

    Note: Participation in an observational clinical study is allowed at the investigator's discretion. Please notify the sponsor (or medical monitor) of this decision.

14. Participant has a history of an underlying clinically significant acute or chronic medical condition or physical examination findings for which, in the opinion of the investigator, participation would not be in the best interest of the participant (eg, compromise the well-being) or that could prevent, limit, or confound the protocol-specified assessments.

15. Participant had major surgery per the investigator's judgement within 12 weeks before vaccination, or will not have fully recovered from surgery, or has a major surgery planned (except for Caesarean Section) within 6 months after the last dose of the study vaccine.

16. Participant has a contraindication to IM injections and blood draws eg, bleeding disorders.

17. Criterion modified per Amendment 1.

    17.1 Criterion deleted per Amendment 2.

    17.2 Criterion reinstated per Amendment 3

    Participant is an employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator, or an employee of the sponsor.

18. Participant has chronic active hepatitis B or hepatitis C infection per medical history.

19. Participant has had major psychiatric illness or drug or alcohol abuse, which in the investigator's opinion would compromise the participant's safety or compliance with the study procedures.

20. Participant cannot communicate reliably with the investigator.

21. Participant who, in the opinion of the investigator, is unlikely to adhere to the requirements of the study or is unlikely to complete the full course of vaccination and observation.

22. Criterion deleted per Amendment 4.

23. Criterion modified per Amendment 2.

    23.1 Participant has a positive diagnostic test result (PCR-based viral RNA detection) SARS-CoV-2 infection at screening or Day 1 (if more than 4 days in between).

24. Participants with medical history of comorbidities that are or might be associated with an increased risk of progression to severe COVID-19. Examples of such conditions are severe asthma; chronic lung diseases (eg, chronic obstructive pulmonary disease [COPD], idiopathic pulmonary fibrosis and cystic fibrosis); diabetes (including type 1, type 2, or gestational); serious heart conditions, including heart failure, coronary artery disease, congenital heart disease, cardiomyopathies, and (pulmonary) hypertension or high blood pressure; pre-pregnancy obesity; severe obesity (BMI $\geq$ 40 kg/m^2); chronic liver disease, including cirrhosis; sickle cell disease; thalassemia; cerebrovascular disease; neurologic conditions; and (unstable/uncontrolled) HIV infection.

    A list of underlying medical conditions that increase the risk of progression to severe COVID-19 for non-pregnant adults is available at the CDC website (at the time of writing this protocol) (CDC 2020b, d) and in Section 10.9.

25. Criterion deleted per Amendment 1

26. Criterion deleted per Amendment 2

27. History of confirmed SARS or MERS

28. Criterion added per Amendment 1

    28.1 Criterion modified per Amendment 4

    28.2 At the time of consenting, participants should agree to practicing an acceptable effective method of contraception postpartum and agree to remain on such a method of contraception following the birth of the infant until 3 months after administration of the last study vaccine. Participants should follow contraceptive (birth control) measures consistent with local regulations regarding the acceptable methods of contraception (see Section 10.5).

29. Criterion added per Amendment 3

    Participant has a history of TTS, including cerebral venous sinus thrombosis (CVST), or heparin-induced thrombocytopenia (HIT).

30. Criterion added per Amendment 3

    Participant has a history of Capillary leak syndrome (CLS).

NOTE: Investigators should ensure that all study enrollment criteria have been met prior to the first dose. If a participant's clinical status changes (including any available laboratory results or receipt of additional medical records) after screening but before the dose of study vaccination and they no longer meet all eligibility criteria, the participant should be excluded from participation in the study. Section 5.4 describes options for rescreening.

The required documentation to support meeting the enrollment criteria is described under Source Documents in Section 10.3.10.

5.3. Lifestyle Considerations

Potential adult participants must be willing and able to adhere to the following lifestyle restrictions during the course of the study to be eligible for participation:

1. Refer to Section 6.8 for details regarding prohibited and restricted therapy during the study.
2. Agree to follow all requirements that must be met during the study as noted in the Inclusion and Exclusion Criteria for adult participants (Section 5.1 and Section 5.2).
3. Adult participants should agree to follow requirements for the electronic completion of the COVID-19 signs and symptoms surveillance questions in the eCOA, as applies to both themselves and to their neonate/infants.

5.4. Screen Failures

Participant Identification, Enrollment, and Screening Logs

The investigator agrees to complete a participant identification and enrollment log to permit easy identification of each participant during and after the study. This document will be reviewed by the sponsor study site contact for completeness.

The participant identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure participant confidentiality, no copy will be made.

All reports and communications relating to the study will identify participants by participant identification and age at initial informed consent. In cases where the participant is not enrolled into the study, the date seen and age at initial informed consent will be used.

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.

5.5. Criteria for Temporarily Delaying Administration of Study Vaccination

The following events constitute a temporary contraindication to study vaccination:

- Clinically significant pregnancy-related conditions according to medical judgement (see Section 7.2).
- Clinically significant acute illness at the time of vaccination. This does not include minor illnesses, such as diarrhea or mild upper respiratory tract infection.
- Fever (body temperature ≥38.0°C [100.4°F]) within 24 hours prior to the planned time of vaccination.

If either of these events occur at the scheduled time for vaccination, then vaccination at a later date within the screening window is permitted at the discretion of the investigator and after consultation with the sponsor. If the vaccination cannot occur within the screening window, rescreening is required. All participants may be rescreened once (see Section 5.4).

The events listed above also apply to the booster vaccination visit for eligible participants. In addition, a urine pregnancy test (for participants of childbearing potential, according to the local guidelines) will be required for the Booster Visit for all participants in Group 4. Participants from Group 4 who are pregnant are not eligible to receive the booster vaccination (see Section 6.2).

If the vaccination visit cannot be rescheduled within the allowed window or the contraindications to vaccination persist, the rationale should be documented.

6. STUDY VACCINATION AND CONCOMITANT THERAPY

6.1. Study Vaccinations Administered

Study Vaccine Information

Ad26.COV2.S will be supplied at a concentration of 1×10^{11} vp/mL, in single-use vials, with an extractable volume of 0.5 mL. Formulation buffer will be supplied as diluent as 15 mM citrate, 5% (w/w) hydroxypropyl-β-cyclodextrin, 0.4% (w/w) ethanol, 0.03% (w/w) polysorbate 80, 75 mM NaCl, pH 6.2,

The vaccine will be extracted from the vial as outlined below:

- Ad26.COV2.S:

  5×10^{10} vp dose level: 0.5 mL is withdrawn from one vial containing 0.75 mL 1×10^{11} vp/mL.

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

Participants will receive a single dose of Ad26.COV2.S at the 5×10^{10} vp dose level, irrespective of gestational age.

The study vaccine will be administered by IM injection into the deltoid muscle, preferably of the non-dominant arm. Subsequent booster vaccination (for Group 4 participants) are preferably administered in the opposite arm. If an injection cannot be given in the deltoid muscle due to a medical or other contraindication (for example, tattooed upper arms rendering it difficult to assess site reactogenicity), alternative locations such as the hip, thigh or buttocks can be used. If alternative locations are used for vaccine administration, the participant's ability to assess injection site events should be considered.

For information on vaccination windows, see Section 8: Study Assessments and Procedures. If a participant cannot be vaccinated within the allowed window (eg, if the window is missed due to a study pause, see Section 6.9), the decision regarding vaccination will be assessed on a case-by-case basis.

Study vaccine administration must be captured in the source documents and eCRF.

Ad26.COV2.S will be manufactured and provided under the responsibility of the sponsor. Refer to the IB (IB 2021) and its addenda for a list of excipients.

Refer to the study site investigational product and procedures manual (SIPPM) and the Investigational Product Preparation Instructions (IPPI) for additional guidance on study vaccine administration.

6.2. Booster Vaccination

Vaccine Naïve Participants

Vaccine naïve participants at study entry (Group 4, including Sentinel and Safety Cohorts) who have completed the pregnancy during which they were enrolled in the study, are not pregnant again will be offered an (optional) booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp. They must not meet any study discontinuation criteria as specified in Section 7.1 and have not received another COVID-19 vaccine (eg, national immunization program) outside the study as indicated in the SoA in Section 1.3.1.1.

The booster vaccination will be administered not earlier than 2 months after completion of the participant's Ad26.COV2.S vaccination in the study. The booster vaccination and the booster follow-up visit should not extend the study duration.

The Booster Vaccination Visit for vaccine naïve participants at study entry should preferably coincide with a scheduled visit from the original SoA in Section 1.3.1. If not operationally feasible to coincide with an existing visit, an unscheduled visit may be planned.

Participants will continue to follow the Schedule of Activities in Section 1.3.1. All participants (whether they consent to the booster vaccination or not) will be encouraged to remain in the study.

Previously Vaccinated Participants (Group 1-3)

Previously vaccinated participants (Groups 1-3) will receive a single (booster) dose of Ad26.COV2.S at 5×10^{10} vp as part of the study, but are not eligible for a second booster during the study.

6.3. Preparation/Handling/Storage/Accountability

Preparation/Handling/Storage

All study vaccine must be stored in a secured location with no access for unauthorized personnel and at controlled temperatures as indicated on the clinical labels. If study vaccine is exposed to temperatures outside the specified temperature range, all relevant data will be sent to the sponsor to determine if the affected supplies can be used or will be replaced. The affected study vaccine must be quarantined and not used until further instruction from the sponsor is received.

Refer to the SIPPM and the IPPI for additional guidance on study vaccine preparation, handling, and storage.

A pharmacist or other qualified individual will prepare the appropriate vial and syringe, labeled with the participant's identification number, and provide the syringe to the vaccine administrator (a trained and qualified study nurse, medical doctor, otherwise qualified HCP) who will perform the injection.

Accountability

The investigator is responsible for ensuring that all study vaccine received at the site is inventoried and accounted for throughout the study. The study vaccine administered to the participant must be documented on the vaccine accountability form. All study vaccine will be stored and disposed of according to the sponsor's instructions. Study site personnel must not combine contents of the study vaccine containers.

Study vaccine must be handled in strict accordance with the protocol and the container label and must be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental conditions. Unused study vaccine must be available for verification by the sponsor's study site monitor during on-site monitoring visits. The return to the sponsor of unused study vaccine will be documented on the vaccine accountability form. When the study site has an authorized destruction unit and study vaccine supplies are destroyed on-site, this must also be documented on the vaccine accountability form.

Potentially hazardous materials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for vaccine accountability purposes.

Study vaccine should be dispensed under the supervision of the investigator or a qualified member of the study site personnel, or by a hospital/clinic pharmacist. Study vaccine will be administered only to participants participating in the study. Returned study vaccine must not be dispensed again, even to the same participant. Study vaccine may not be relabeled or reassigned for use by other participants. The investigator agrees neither to dispense the study vaccine from, nor store it at, any site other than the study sites agreed upon with the sponsor. Further guidance and information for the final disposition of unused study vaccine are provided in the SIPPM.

Measures to Minimize Bias: Vaccine Allocation

Sentinel and Safety Cohorts participants received 1 dose of Ad26.COV2.S at 5×10^{10} vp. At the time of writing Amendment 5, 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 following review of the Sentinel and Safety Cohorts safety data. The remaining participants will receive 1 dose of Ad26.COV2.S at 5×10^{10} vp.

Participants will be stratified by pregnancy stage (Weeks ≥16 to <28 or Weeks ≥28 to ≤38). Efforts will be made to enroll at least approximately 25% per trimester, per group. The assignment will utilize a computer-generated schedule prepared before the study under the sponsor's oversight.

Participants who were vaccine naïve at study entry will be offered a booster dose of Ad26.COV2.S at 5×10^{10} vp during the study.

Blinding

This is an open-label study, therefore, blinding procedures are not applicable.

6.4. Study Vaccination Compliance

Study vaccines will be administered IM by qualified study site personnel at the study site per local regulations. Details of each administration will be recorded in the eCRF (including date and time of injection, and the location used for injection [deltoid or alternative location]).

6.5. Dose Modification

The safety and tolerability of a single dose of the Ad26.COV2.S vaccine at 5×10^{10} vp in adult participants was assessed in Sentinel and Safety Cohorts.

At the time of writing Amendment 5, 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 following review of the Sentinel and Safety Cohorts safety data.

The remaining participants will receive 1 dose of Ad26.COV2.S at 5×10^{10} vp dose level.

6.6. Continued Access to Study Vaccine After the End of the Study

Not applicable.

6.7. Treatment of Overdose

For this study, any dose of Ad26.COV2.S greater than the assigned dose will be considered an overdose. The sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the investigator should:

- Contact the relevant sponsor or delegate immediately.
- Closely monitor the participant for AEs/SAEs/pregnancy-related AEs/MAAEs (ie, the participant will remain at the study site for at least 1 hour and will be closely monitored for allergic or other reaction by study staff. Follow-up telephone calls 12 hours and 24 hours post-vaccination can be made).
- Document the quantity of the excess dose in the eCRF.
- Report as a special reporting situation.

6.8. Prestudy and Concomitant Therapy

Prestudy therapies such as analgesic/antipyretic medications and non-steroidal anti-inflammatory drugs, corticosteroids, and antihistamines used 30 days before administering the study vaccine must be recorded.

Concomitant therapies such as analgesic/antipyretic medications and non-steroidal anti-inflammatory drugs, corticosteroids, antihistamines, and vaccinations must be recorded (including dose and frequency) from study vaccination until 28 days after administration of study vaccine. All other concomitant therapies should also be recorded if administered in conjunction with a confirmed COVID-19 case or with new or worsening AEs or suspected AESI reported per protocol requirements outlined in Section 8.3.1.

Concomitant therapies associated with an SAE meeting the criteria outlined in Section 10.4.1 will be collected and recorded in the eCRF from study vaccination through the end of the study for adult participants and from birth until the end of the study for neonates/infants. Concomitant therapies associated with MAAEs will be collected and recorded in the eCRF from study vaccination until 6 months after vaccination for adults and from birth until 6 months of age for neonates/infants. Concomitant therapies associated with AEs leading to study discontinuation will be recorded in the eCRF during the entire study for both adult and neonates/infants.

Use of any investigational medication (including experimental vaccines other than the study vaccine) during the study, other than the study vaccine, will lead to discontinuation of administration of any subsequent study vaccination. Participants may not receive:

- an investigational drug or use an invasive investigational medical device within 30 days, or
- investigational immunoglobulin or monoclonal antibodies within 3 months, or
- convalescent serum for COVID-19 treatment within 4 months, or
- an investigational vaccine (including investigational Adenoviral-vectored vaccines) within 4 months before the planned administration of study vaccine.

During the study, the use of investigational COVID-19 vaccines other than the study vaccine is not allowed, and the use of investigational drugs is only allowed if medically indicated. In the event that a participant receives another COVID-19 vaccine outside of the study, investigators are required to enter any COVID-19 vaccines (name/manufacturer of the vaccine and date of administration) in the Concomitant Therapy eCRF.

Treatment with investigational COVID-19 drugs after diagnosis of a COVID-19 case is allowed during the postpartum follow-up period and needs to be recorded in the COVID-19 episode description.

Licensed live attenuated vaccines are generally contraindicated during pregnancy. Licensed (not live) vaccines (eg, the DtaP [reduced Diphtheria toxoid, Tetanus toxoid, and acellular Pertussis], influenza, tetanus, hepatitis A, hepatitis B, rabies vaccines) should be given at least 14 days before or at least 14 days after administration of study vaccine in order to avoid potential confusion of adverse reactions and potential immune interference, and must be recorded. The use of any coronavirus vaccine (licensed or investigational) is disallowed at any time prior to vaccination except for those licensed vaccines indicated in inclusion criteria. The use of any coronavirus vaccine (licensed or investigational) is disallowed during the study, and must be reported in EDC. If a vaccine is indicated in a post-exposure setting (eg, rabies or tetanus), it should take priority over the study vaccine, and any vaccinations must be recorded.

All vaccines administered to the babies during the study should be captured.

Treatment with immunoglobulins and blood products is disallowed in the 3 months and 4 months before the planned administration of study vaccine, respectively, and during the study. Immunoglobulin Rho (D) can be given at any time.

Antipyretics (eg, acetaminophen/paracetamol) after vaccination, and additional doses spaced 6 hours apart may be administered thereafter for symptom relief as needed. Prophylactic antipyretic use is not encouraged; however, it could be considered per investigator's discretion.

Chronic (daily for 2 consecutive weeks) or recurrent use of systemic corticosteroids (>20 mg/day of prednisone or equivalent)[a] at immunosuppressive doses and administration of antineoplastic and immunomodulating agents or radiotherapy are prohibited during the study and within 6 months before the planned administration of study vaccine. If any of these agents are indicated in a disease setting, treatment takes priority over the study vaccine.

Refer to Section 5.2 for further details of prohibited therapy.

The sponsor must be notified as soon as possible of any instances in which prohibited therapies are administered. Depending on the time of the occurrence, any participant who receives a prohibited concomitant medication will not be included in the immunogenicity analyses.

6.9. Study Vaccination Pausing Rules

The Sponsor (including designated sponsor teams) and/or Sponsor Committee as well as the Investigator(s) will monitor safety, including the study vaccination pausing rules.

If a study vaccination is considered to raise significant safety concerns (and a specific pause rule has been met), further vaccination of participants will be paused.

The IDMC will review all available safety data and make recommendations regarding the continuation of the study to the sponsor study team. The formal recommendation from IDMC will be provided by the sponsor to applicable health authorities.

The occurrence of any of the following events will lead to a pause in further study vaccination:

1. Death of an adult participant, considered related to study vaccine or if the causal relationship to the study vaccine cannot be excluded; OR
2. One or more adult participants experience an SAE, a suspected AESI or a Grade 4 (solicited or unsolicited) AE or a persistent (upon repeat testing) Grade 4 laboratory abnormality that is determined to be related to study vaccine; OR
3. Three or more adult participants experience a Grade 3 unsolicited AE of the same type (per medical judgement of the sponsor), that is determined to be related to study vaccine; OR
4. Three or more adult participants experience a persistent (upon repeat testing) Grade 3 laboratory abnormality related to the same laboratory parameter and considered related to study vaccine; OR

[a] Note: Ocular, topical or inhaled steroids are allowed.

5. Three or more adult participants experience a Grade 3 solicited AE of the same type, determined to be related to study vaccine, and persisting as Grade 3 for longer than 3 consecutive days (ie, the day of occurrence of the AE is counted as Day 1). [a]

For number 2 and number 4: to assess abnormal laboratory values, the test must be repeated at least once, within 48 hours of the site becoming aware of the abnormal value.

For number 2, number 3, and number 5: after each IDMC review of similar AEs, the Committee will indicate the conditions under which it requires further notification and review of any subsequent similar AEs.

Note: the occurrence of a study pause in any other ongoing study with Ad26.COV2.S may trigger a pause in further vaccination in the current study, if considered to be medically relevant. Any subsequent study visits as a result of a delayed vaccination following a study pause are not considered to be protocol violations, however, the data at these timepoints may be eliminated from certain statistical analyses.

Based on the pausing criteria, the sponsor will notify other sites in case of a study pause. To enable prompt response to a situation that could trigger pausing rules, the investigator should notify the sponsor, immediately and no later than 24 hours after becoming aware of any related AE of Grade 3 or above and update the eCRF with relevant information on the same day the AE information is collected.

A thorough analysis of all Grade 3 (or above) cases will be carried out by the sponsor, irrespective of whether the criteria for pausing the study are met. Based on the pausing criteria, the sponsor will notify other sites in case of a study pause. The sponsor's medical monitor or designee is responsible for notification of IDMC members in case a study pause is declared.

Vaccinations for an individual participant may be suspended for safety concerns other than those described in the pausing criteria, at the discretion of the investigator if he/she feels the participant's safety may be threatened. The sponsor may initiate IDMC review for any single event or combination of multiple events which, in their professional opinion, could jeopardize the safety of the participants or the reliability of the data.

Resumption of vaccinations will start upon receipt of written recommendations by the IDMC. If any pausing rule is met (refer to Section 10.3.6 Safety Monitoring Committee Structure) and, if following appropriate safety review it is deemed appropriate to restart dosing, the sponsor will comply with regulatory requirements per competent authority(ies).

The clinical site(s) will be allowed to resume activities upon receipt of a written notification from the sponsor. These communications from the IDMC will be forwarded to relevant parties according to local regulations.

[a] The day of occurrence of the AE is counted as Day 1.

7. DISCONTINUATION OF STUDY VACCINATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Vaccination

Study vaccinations will be withheld for the reasons listed below. These participants could remain on the study for safety and immunogenicity follow-up. Additional unscheduled visits may be performed for safety reasons, if needed. In case of questions, the investigator is encouraged to contact the sponsor.

A participant's study vaccination must be discontinued if:

- Any serious related AE, worsening of health status or intercurrent illnesses that, in the opinion of the investigator, requires discontinuation from study vaccine.
- Anaphylactic reaction following vaccination, not attributable to causes other than vaccination.
- SAE or other potentially life-threatening (Grade 4) event that is determined to be related to study vaccine.
- Chronic (daily for 2 consecutive weeks) or recurrent use of systemic corticosteroids (>20 mg/day of prednisone or equivalent)[a] and administration of antineoplastic and immunomodulating agents or radiotherapy.
- Withdrawal of consent to receive further study vaccination.
- Participant receives a experimental medication (including vaccines during the study) or receives a COVID-19 vaccine outside the study.
- Participant who had previously experienced TTS or HIT.
- Participant who has previously experienced CLS or GBS.

7.2. Delay or Discontinuation of Vaccination due to Pregnancy-Specific Complications

The booster vaccination (Group 4 only) (see Section 6.2) may be withheld or delayed if the participant experiences any of the following pregnancy-specific complications prior to the scheduled booster vaccination:

- severe vaginal bleeding;
- preterm premature rupture of membranes;
- severe pre-eclampsia, eclampsia;
- hemolysis elevated liver enzymes and low platelets (HELLP) syndrome;
- stillbirth;
- fetal loss;

[a] Note: Ocular, topical or inhaled steroids are allowed.

- preterm birth;
- participant has a positive diagnostic molecular test result for SARS-CoV-2 infection on the day of the booster vaccination.

In the event that any of the above occurs, the participant (and infant, if applicable) will continue to be followed until the end of the study in accordance with the Schedules of Activities.

7.3. Participant Discontinuation/Withdrawal From the Study

A participant will be withdrawn from the study for any of the following reasons:

- Lost to follow-up
- Withdrawal of consent
- Death
- Repeated failure to comply with protocol requirements

When a participant withdraws before study completion, the reason for withdrawal is to be documented in the eCRF and in the source document. If the reason for withdrawal from the study is withdrawal of consent, then no additional assessments are allowed.

For those participants who are unable to continue participation in the study up to the last planned study visit, but for whom consent is not withdrawn, an early exit visit will be conducted as soon as possible. Participants who wish to withdraw consent from participation in the study will be offered an optional visit for safety follow-up. This includes the safety assessments of the early exit visit (no blood sampling for immunogenicity).

Withdrawal of Consent

A participant declining to return for scheduled visits does not necessarily constitute withdrawal of consent. Alternate follow-up mechanisms that the participant agreed to when signing the consent form apply as local regulations permit.

7.3.1. Withdrawal From the Use of Research Samples

Withdrawal From the Use of Samples in Future Research

The participant may withdraw consent for use of samples for research (refer to long-term retention of samples for additional future research in Section 10.3). In such a case, samples will be destroyed after they are no longer needed for the clinical study. Details of the sample retention for research are presented in the main ICF.

7.4. Lost to Follow-up

To reduce the chances of a participant being deemed lost to follow-up, attempts should be made to obtain contact information from each participant, eg, home, work, and mobile telephone numbers and e-mail addresses for both the participant as well as appropriate family members. A participant (adult, neonate, and infant) will be considered lost to follow-up if they repeatedly fails

to return for scheduled visits and is unable to be contacted by the study site after multiple documented attempts.

A participant (adult, neonate, and infant) cannot be deemed lost to follow-up until all reasonable efforts made by the study site personnel to contact the participant are deemed futile. The following actions must be taken if a participant fails to return to the study site for a required study visit:

- The study site personnel must attempt to contact the participant to reschedule the missed visit as soon as possible, to counsel the participant on the importance of maintaining the assigned visit schedule, to ascertain whether the participant wishes to or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every reasonable effort to regain contact with the participant (where possible, 3 telephone calls, e-mails, fax, and, if necessary, a certified letter to the participant's last known mailing address, or local equivalent methods). Locator agencies may also be used as local regulations permit. These contact attempts should be documented in the participant's medical records.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study.

Should a study site close, eg, for operational, financial, or other reasons, and the investigator cannot reach the participant to inform them, their contact information may be transferred to another study site.

8. STUDY ASSESSMENTS AND PROCEDURES

Overview

The Schedules of Activities (Section 1.3) summarize the frequency and timing of safety, reactogenicity, immunogenicity, and other measurements applicable to this study. All participants or parent(s)/caregiver(s) (in case of neonates/infants) in the study will be counselled on COVID-19 infection at any interaction with study site staff in line with local guidelines.

All adult participants will be provided access to an eCOA. The eCOA device will be used to collect (suspected) COVID-19 signs and symptoms surveillance information (Symptoms of Infection with Coronavirus-19 [SIC], including body temperature, and pulse oximetry results) at baseline and in case of COVID-19-like signs and symptoms.

All eCOA assessments should be conducted/completed before any tests, procedures, or other consultations to prevent influencing participant responses. Parents/caregivers should also perform (suspected) COVID-19 surveillance (symptom check) and record any signs or symptoms that are suggestive of possible COVID-19 for the neonate/infant and complete the ObsRO for the Pediatric Symptoms of Infection with Coronavirus-19 [PedSIC], including the highest body temperature recorded within 24 hours, blood oxygen saturation levels, and pulse rate, in the eCOA. Procedures for adult participants and neonates/infants with (suspected) COVID-19 are outlined in Section 1.3.4 and Section 1.3.5, respectively.

Adult participants will be provided with a thermometer (to measure body temperature), ruler (to measure local injection site reactions) to record body temperature and solicited local (at injection site) and systemic signs and symptoms in the eCOA device. Parent(s)/caregiver(s) will be provided with a separate thermometer for the neonate/infant to record body temperature for the neonate/infant in the eCOA, in the event of confirmed or suspected COVID-19.

The site staff will be provided with ePRO and ObsRO completion guidelines that includes instructions on capturing responses in the eCOA and grading scales to assess severity of the signs and symptoms after vaccination (reactogenicity). The study staff is responsible for providing appropriate training to the participant/parent(s)/caregiver(s) to prevent missing or incorrect data. The reactogenicity diary will be reviewed by the study personnel at the Day 8 visits indicated in the Schedule of Activities (Section 1.3.2 and Section 1.3.3, respectively). If the reactogenicity diary review is missed/unavailable at Day 8, the reactogenicity diary will be reviewed during the following visit.

Sites should reach out to a participant if the participant fails to complete any of the questions displayed in the eCOA. The questionnaire will be accessible on the eCOA platform in between scheduled reminders and participants will be encouraged to answer the surveillance question in the eCOA as soon as possible after the onset of COVID-19-like symptoms, or any other symptoms with new onset, including those listed above. Every effort will be made to document the status of all participants that are lost to follow-up due to not completing the eCOA and for whom hospitalization has not been recorded.

If multiple assessments are scheduled for the same timepoint, it is recommended that procedures be performed in the following sequence: (a) for adult participant: physical/obstetric examination and vital signs, ultrasound blood draws, and vaccination; (b) for neonate/infant: physical examination and vital signs, blood draws. If needed, assessments may be performed at another day within the applicable visit window. Actual dates and times of assessments will be recorded in the source documentation and eCRF.

Adult participants will also be provided with a kit to collect nasal swabs at Day 1 to be used in case they experience COVID-19-like symptoms during the study (see Section 8.1.2). In the event of the neonate/infants developing COVID-19 like symptoms, the parent(s)/caregiver(s) should contact the site, who will provide a trained HCP to collect nasal swabs from the neonate/infant.

If allowed by local regulations, study visits other than screening and vaccination visit may take place at other locations if there are travel restrictions in case of exceptional situations (eg, pandemic).

Blood Collection - Adult Participants

The maximum total blood volume (without blood samples for PBMCs) to be collected over the course of the study is approximately 166 mL from adult participants receiving 1 dose of Ad26.COV2.S at 5×10^{10} vp.

The maximum total blood volume collected over the course of the study including blood samples for isolation of PBMC for assessment of cellular immunity is approximately 256 mL from adult participants who were enrolled in the PBMC subset of the study.

An additional 60 mL of blood will be collected from participants who receive the booster vaccination.

Further information is provided summarized in Table 1.

Table 1: Maximum Volume of Blood to be Collected from Each Participant

Type of Sample	Volume per Sample (mL)	No. of Samples per Participant 1-dose	Approximate Total Volume of Blood (mL)[a] 1-dose
Adults			
Safety laboratory assessments (hematology, chemistry)	6.5	Max 7	Max 45.5
Humoral Immunogenicity (serum), serology testing (SARS-CoV-2 Abs)	10	4	40
Serological Analysis of anti-SARS-CoV-2 antibodies	2.5	4	10
Plasma collection (safety+immunogenicity)	5	5	25
Serum collection (safety)	7.5	5	37.5
Biomarkers vaccine-related (whole peripheral blood) PAXgene®	2.5	3	7.5
Cellular Immunogenicity (PBMC) subset	30	3	90
Approximate total for adults excluding participants in the PBMC subset			**Max 165.5**
Approximate total for subset of adults with blood for PBMC collection			**Max 255.5**
Adults - additional blood draws in case of (suspected) COVID-19			
Humoral Immunogenicity (serum)	10	2	20
Biomarkers PAXgene®	2.5	2	5
Adults - additional blood draws in case of (suspected) AESI			
Clinical laboratory blood sample (whole blood)	15	2	30
Neonates and infants			
Humoral Immunogenicity (serum) ≤6 months of age	3.5	2	7
Approximate total for infants			**7**
Neonates and infants - additional blood draws in case of (suspected) COVID-19			
Humoral Immunogenicity (serum)	2.5	2	**5**
Neonates cord blood			
Humoral Immunogenicity (cord blood)	15[b]	1	15[c]
Biomarkers (cord blood) PAXgene®	2.5	1	2.5
Approximate (minimum) total			**17.5**
Adults - Additional blood draws in case of receiving the booster vaccination			
Clinical blood sample (safety, whole blood)	15	2	30
Humoral Immunogenicity (serum)	10	2	20
Serological Analysis of anti-SARS-CoV-2 antibodies	2.5	2	5
Biomarkers vaccine-related (whole peripheral blood) PAXgene®	2.5	2	5
Approximate additional total in case of receiving the booster vaccination			**60**

a. Calculated as number of samples multiplied by amount of blood per sample. Repeat or unscheduled samples may be taken for safety reasons or technical issues with the samples.
b. Approximately 15 mL (minimum of 10 mL).

Visit Windows

Visit windows that will be allowed are provided in Section 1.3 and summarized below. The participant should be encouraged to come on the day planned but using the visit window is allowed. The timings of the post-vaccination visits will be determined relative to the actual day of the corresponding vaccination. If a participant misses a vaccination or has a delayed vaccination, the post-vaccination visits will be calculated from the imputative vaccination date according to protocol.

If a vaccination window is missed, efforts will be made to vaccinate and collect samples from the participant, even if out of window. The timings of the post-vaccination visits will be determined relative to the actual day of the vaccination, unless they overlap with other scheduled visits. If so, it would be evaluated in a case-by-case basis in which case the sponsor should be contacted, and a case-by-case assessment agreed.

Screening

Screening will be performed within 28 days prior to study vaccination or on the day of vaccination. If screening is performed on the day of vaccination, study visits will be combined (ie, for adult participants, Visits 1 and 2 will occur on Day 1). Screening must be completed, and all eligibility criteria must be fulfilled prior to vaccination. Participants may be rescreened only once (see Section 5.4).

The nasal swab for the RT-PCR test for the presence of SARS-CoV-2 infection must be done within 4 days before vaccination and repeated pre-vaccination if necessary. The study-specific ICF date will be entered into the eCRF.

Sample Collection and Handling

The actual dates and times of sample collection must be recorded in the eCRF or laboratory requisition form. Refer to Section 1.3 for the timing and frequency of all sample collections.

Instructions for the collection, handling, storage, and shipment of samples can be found in the laboratory manual that will be provided. Collection, handling, storage, and shipment of samples must be under the specified, and where applicable, controlled temperature conditions as indicated in the laboratory manual.

Study-Specific Materials

The investigator will be provided with the following supplies:

- Latest version of the Ad26.COV2.S Investigator Brochure and Addenda
- Thermometer (for adults and neonates/infants)
- Ruler (to measure diameter of any erythema and swelling)
- An approved pulse oximeter (for adults and neonates/infants)
- Pharmacy manual/SIPPM

- Laboratory manual
- IWRS Manual
- eCRF completion guidelines
- Sample ICF
- Nasal swab kit, and participants instructions
- eCOA platform access and participant instructions (participants may use their own eDevice using an application if their device [smartphone or tablet] is compatible, or a web portal). Provisioned devices will be available on a limited basis. Adult participant reactogenicity diaries and the latest Independent Ethics Committee/Institutional Review Board (IEC/IRB)-approved versions of the PedSIC/SIC are part of the eCOA
- Contact information page(s)
- Tablet for eConsent, if applicable

8.1. Immunogenicity Assessments

8.1.1. Immunogenicity Assessments

Blood Sampling

From all adult participants (venous blood), neonates (cord blood at birth), and infants (venous or arterial blood), blood samples will be collected at selected timepoints for humoral immunogenicity assessments, with an emphasis on neutralizing and binding antibody responses. In addition, blood samples will be collected at selected timepoints, analysis of vaccination-related biomarkers in PAXgene® tubes may be performed. From a subset of approximately 25 adult participants per group, PBMCs was planned to be collected for analysis of cellular immunogenicity (including Th1/Th2 assessments). With only 1 participant in this subset at the time of writing this Protocol Amendment 7, PBMC sampling will be removed from the study as of Protocol Amendment 7 approval.

For participants who receive a booster vaccination, blood samples will be collected from adults pre-boost and 28 days post-boost for humoral immunogenicity assessments and vaccination-related biomarkers.

Sample volumes and time points for adult participants and neonates/infants are detailed in the Schedules of Activities in Section 1.3, and outlined below.

Humoral and cellular immunogenicity assays may include, but are not limited to, the assays summarized in Table 2.

Table 2: Summary of Humoral and Cellular Immunogenicity Assays

Summary of Humoral Immunogenicity Assays	
Assay	**Purpose**
Primary/Secondary endpoints	
SARS-CoV-2 binding antibodies (ELISA or equivalent assay)	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, 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
SARS-CoV-2 binding immunoglobulins, including IgA antibodies (ELISA or equivalent assay)	Analysis of IgA and/or other Ig subtypes against SARS-CoV-2 in colostrum and/or breast milk
Adenovirus 26 neutralization (neutralization assay)	Analysis of neutralizing antibodies to Adenovirus 26
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 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

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 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; Th1/Th2:T helper cell subset(s); TNFα tumor necrosis factor alpha; VNA virus neutralization assay.

Colostrum and Breast Milk Sampling

Colostrum (approximately 0.5 mL) should be collected within 1 week postpartum and breast milk (≥5 mL) at Day 43 (±14 days) during the postpartum follow-up phase, if feasible, from participants who have received Ad26.COV2.S to assess the presence of immunoglobulins, including IgA against SARS-CoV-2 (see Schedules of Activities in Section 1.3.2 and Section 1.3.3). Colostrum and/or breast milk will also be collected, if feasible, at the time of booster vaccination and 28 days post booster vaccination, in participants who were vaccine naïve at study entry and receive a booster after pregnancy completion.

8.1.2. Procedures in the Event of (Suspected) COVID-19

Procedures to be performed in the event a participant (adult or neonate/infant) experiences signs or symptoms suggesting possible COVID-19, or a participant became aware of a RT-PCR test result for SARS-CoV-2 outside the study context, whether symptomatic or asymptomatic, are detailed in the Schedule of Activities in Section 1.3.4 (adults) and Section 1.3.5 (neonates/infants).

Note: if a clinic visit is not feasible a home may be allowed if allowed per local regulations.

If site staff are unable to perform home visits, a trained and delegated HCP may perform the visits and assessments described below if allowed by local regulations. If a home visit is not feasible, procedures and activities may be done by other methods where possible (such as telephone or video conferencing). The principal investigator (PI) continues to be responsible for reviewing all protocol-related assessments. Site staff/HCP visiting participants at home will use personal protective equipment according to local regulation.

In case the participant experiences any signs or symptoms suggesting possible COVID-19, medical management of the case should follow local recommendations/guidelines.

8.1.2.1. Procedures for Adult Participants in Case of Signs and Symptoms of COVID-19

Day 1-2 Procedures in Case of Signs and Symptoms

If an adult participant (or their designated caregiver) records in the eCOA or informs the site that she experienced any signs or symptoms suggesting possible COVID-19, this will be considered **COVID-19 Day 1** (day of onset of signs and symptoms). The adult participant will be asked to complete the ePROs (ie, the SIC, including body temperature) in the eCOA.

Notes:

The SIC questionnaire asks the adult participant if she had any of the prespecified signs or symptoms during the past 24 hours, and (when applicable) to rate the severity. The SIC questionnaire takes approximately 5 minutes to complete.

The adult participant should record the highest temperature in the last 24 hours in the SIC.

The adult participant should record 1 of the 3 pulse oximetry readings in the last 24 hours in the eCOA.

If an adult participant is unable to complete the SIC in the eCOA, the site can collect information on the participant's symptoms and body temperature, by contacting the participant by telephone (or visit the participant at home), reading the questions aloud to the participant and entering the participant's responses on the participant's behalf. More details are provided in the eCOA Study Manual.

If a participant is unable to complete the SIC in eCOA, the reason for missing the SIC completion should be recorded in the eCRF.

Based on the information collected through the SIC, the site will reach out to the adult participant at the latest on COVID-19 Day 2 (the day after the day of symptom onset) to assess whether the reported signs and symptoms qualify as a suspected COVID-19 episode using prespecified criteria (Section 8.1.2.4). As several of the prespecified criteria for suspected COVID-19 overlap with vaccine-related reactogenicity, investigators' clinical judgement is required to exclude vaccine-related events when assessing suspected COVID-19. If the adult participant would actively reach out to the site already on COVID-19 Day 1, the site should already make a first assessment on COVID-19 Day 1 to check whether the reported signs and symptoms qualify as a suspected COVID-19 episode using prespecified criteria (Section 8.1.2.4). As soon as the prespecified criteria for suspected COVID-19 are met (**COVID-19 Day 1-2**), the adult participant will be asked to undertake the COVID-19 procedures. In particular:

The adult participant will be asked to continue to complete the ePROs in the eCOA as specified above for COVID-19 Day 1:

- SIC (including body temperature): every day, preferably in the evening around the same time each day. If more than one measurement is made on any given day, the highest temperature of that day will be recorded.

- Blood oxygen saturation and pulse rate using a pulse oximeter 3 times a day, preferably in the morning, at lunch time, and in the evening.

  Note: the ePROs do not have to be completed if special circumstances occur, such as hospitalization or ventilation, in which case the reason for not completing the ePROs should be recorded by site staff in the eCRF.

The adult participant will be asked to collect a nasal swab at home on **COVID-19 Day 1-2**, as soon as possible after it has been confirmed that the prespecified criteria for suspected COVID-19 are met. If the adult participant requires assistance, a trained HCP can help the participant to collect the nasal swab. The study site should arrange transfer of the nasal swab to the study site as soon as possible after collection, preferably within 3 days. The COVID-19 Day 1-2 nasal swab can also be collected at the study site (or hospital or other location, if needed), if preferred by the adult participant.

Day 1-2 Procedures in Case of a Positive RT-PCR Test Outside the Study Site Context

If an adult participant becomes aware of a positive RT-PCR test for SARS-CoV-2 she should contact the site as soon as possible. The day the adult participant became aware of the positive PCR test will be considered **COVID-19 Day 1**. Regardless of whether the adult participant is symptomatic or asymptomatic, they will be asked to:

Complete the (suspected) COVID-19 surveillance (symptom check) in the eCOA. In case of COVID-19-like signs and symptoms they will need to complete the SIC (including body temperature) in the eCOA.

The adult participant will be asked to collect a nasal swab at home on **COVID-19 Day 1-2**, as described for the participants with signs and symptoms (see above).

If a participant has a positive test result for SARS-CoV-2 infection, if necessary, study-site personnel may visit the participant at home[a]. The participant will be contacted by the site at least once per week. The SIC will be reviewed by study staff during these contacts (phone call or visit).

Day 3-5 Procedures for all Adult Participants Who Have met the Prespecified Criteria for (Suspected) COVID-19

The adult participant will be asked to come to the site on **COVID-19 Day 3-5** (between 2 and 4 days after symptom onset/becoming aware of a positive RT-PCR test).

If a site visit is not feasible, a member of the study staff could visit the participant at home (or at the hospital or other location, if needed), if allowed by local regulations. The study staff visiting participants at home will use personal protective equipment according to local regulations. The COVID-19 Day 3-5 assessments may also be performed by a trained HCP, if allowed per local regulations.

During **Part 1** of the **COVID-19 Day 3-5** visit, if the adult participant has experienced COVID-19 like signs and symptoms, the site will interview the participant to assess whether the reported signs and symptoms still qualify as a suspected COVID-19 episode

[a] The study staff visiting participants at home will use personal protective equipment according to local regulations.

using prespecified criteria (Section 8.1.2.4). In addition, for all participants with (suspected) COVID-19, a qualified member of the study site will measure vital signs (body temperature, blood pressure, heart rate, and respiratory rate) and pulse oximetry. A targeted physical examination will be performed based on the judgement of the investigator or designated medically qualified healthcare professional. A nasal swab will be collected for detection of SARS-CoV-2 by a qualified member of the study site.

If the prespecified criteria for suspected COVID-19 are still met on COVID-19 Day 3-5 or if at least one nasal swab from COVID-19 Day 1-2 or Day 3-5 visits is positive for SARS-CoV-2 (tested by RT-PCR), the following assessments and procedures are to be performed during **Part 2** of the **COVID-19 Day 3-5** visit: blood samples for exploration of biomarkers that correlate with SARS-CoV-2 infection and COVID-19 severity and for exploration of antibody responses to infection with SARS-CoV-2 will be collected by a qualified member of the study site and the procedures for the 7-day cycles should be started (see below).

The medical history (general and obstetric) and description of COVID-19 episode will be collected by interview with the participant.

If signs and symptoms are still ongoing on COVID-19 Day 3-5, collection of SIC will continue as specified in the next section (Closure of the COVID-19 episode).

If the prespecified criteria for suspected COVID-19 are no longer met on COVID-19 Day 3-5, the participant will not undertake any further COVID-19 procedures. She will fall back to the default Schedule of Activities.

Procedures During the 7-day Cycles

If an adult participant meets the prespecified criteria at Day 3-5 (even if nasal swab results from Day 1-2 and Day 3-5 are pending) and/or has at least one positive nasal sample for SARS-CoV-2 at COVID-19 Day 1-2 or COVID-19 Day 3-5, she will be asked to undertake the COVID-19 procedures, in particular:

In case of signs and symptoms: The participant will be reminded to further complete the ePROs in the eCOA as described for COVID-19 Day 1-2:

In case the nasal swabs collected at Day 1-2 or Day 3-5 visits are tested positive for SARS-CoV-2 and the participant is asymptomatic: The participant will be reminded to further complete (suspected) COVID-19 surveillance (symptom check, at least twice a week).

All participants will be asked to collect a nasal swab at home once every 7 days. If the participant requires assistance, a trained HCP can help the participant to collect the nasal swabs. The study site should arrange transfer of the nasal swabs to the study site within 3 days after collection. Details are provided in the laboratory manual.

If, due to lack of signs and symptoms and unavailability of results from nasal swabs collected on Day 1-2 and/or Day 3-5 visits, the participant stopped the COVID-19 procedures and returned to default Schedule of Activities on COVID-19 Day 3-5, the

participant will be contacted as soon as at least one of these samples is found to be positive for SARS-CoV-2 The participant will be asked to resume COVID-19 procedures until 14 days after symptom onset (COVID-19 Day 15) or until resolution of the COVID-19 episode, whichever comes last.

Note: If results of both Day 1-2 and Day 3-5 nasal swabs once available are negative, the participant will stop the 7-day cycle and fall back to the default Schedule of Activities.

Note: Participants should be encouraged by the site to collect nasal swabs as indicated in Section 1.3.4. If the participant is unable or unwilling to collect all samples as requested, the participant should still complete the other COVID-19 assessments, including the visit at COVID-19 Day 29.

COVID-19 Day 29 Procedures

If an adult participant has at least 1 SARS-CoV-2 positive nasal swab collected on COVID-19 Day 1-2 or Day 3-5, then she will be asked to return to the site on COVID-19 Day 29 (±7 days) where a blood sample will be drawn for sero-confirmation and exploration of biomarkers that correlate with SARS-CoV-2 infection and COVID-19 severity. A qualified member of the study site will measure vital signs (body temperature, blood pressure, heart rate, and respiratory rate) and pulse oximetry. A targeted physical examination will be performed based on the judgement of the investigator or designated medically qualified healthcare professional. The medical history (general and obstetric) and description of COVID-19 episode will be collected by interview with the participant. If the participant is still symptomatic, she will complete the SIC in the eCOA. Asymptomatic participants will complete the (suspected) COVID-19 surveillance (symptom check).

Notes: COVID-19 Day 29 procedures should still be performed even if the nasal swabs results are still pending. The COVID-19 Day 29 assessments may also be performed by a trained HCP at the participant's home, if allowed per local regulations.

This visit can be combined with a regular study visit if within the applicable visit windows.

Closure of the COVID-19 Episode

The adult participant should continue the COVID-19 procedures until any of the following occurs, based on molecular test results:

If both nasal swabs (collected on COVID-19 Day 1-2 and COVID-19 Day 3-5) are **negative** for SARS-CoV-2, the participant will not undertake any further COVID-19 procedures and will fall back to the default Schedule of Activities.

If the participant has at least 1 SARS-CoV-2 positive nasal swab collected on COVID-19 Day 1-2 or Day 3-5, then the participant will be asked to undertake the COVID-19 procedures until 14 days after symptom onset (COVID-19 Day 15, if applicable) or until

resolution of the COVID-19 episode, whichever comes last[a]. Resolution of the COVID-19 episode is defined as having 2 consecutive SARS-CoV-2 negative nasal swabs and 2 consecutive days with no COVID-19-related signs or symptoms. Once past COVID-19 Day 15, participants should stop the collection of nasal swabs as soon as 2 consecutive nasal swabs are SARS-CoV-2 negative, but (if still symptomatic at that time) should continue completing the ePROs (including SIC, body temperature, and pulse oximetry) in the eCOA until 2 consecutive days with no COVID-19-related signs or symptoms.

If signs and symptoms are still ongoing on COVID-19 Day 3-5, collection of SIC will be continued until at least 14 days after onset unless both COVID-19 Day 1-2 and COVID-19 Day 3-5 nasal swabs are negative. If either of the swabs is positive or the result is unknown AND the participant is beyond 14 days after onset of symptoms, the SIC can be stopped after 2 days without signs and symptoms.

For participants with a positive test result for SARS-CoV-2 infection, a study visit will be conducted 28 days after symptom onset (ie, at the COVID-19 Day 29 visit) to assess the clinical course of the infection.

If 2 consecutive nasal swabs negative for SARS-CoV-2 are not available due to operational reasons (eg, delays in results availability), participants may cease collection of nasal swabs samples at the COVID-19 Day 29 visit, provided they have 2 consecutive days with no COVID-19-related signs and symptoms. In these cases, participants may be asked to resume sample collection if nasal sample results once available do not present with 2 consecutive negative swabs for SARS-CoV-2.

Upon closure of the COVID-19 episode and procedures, all adult participants will fall back to the default Schedule of Activities[b] (Section 1.3.2).

If the adult participant experiences new signs or symptoms suggesting possible COVID-19 at a later point in time, the participant would restart the COVID-19 procedures from COVID-19 Day 1 onwards.

8.1.2.2. Procedures for Neonate and Infant Participants in Case of Signs and Symptoms of COVID-19

The following procedures are specific for the study:

Day 1-3 Procedures in Case of Signs and Symptoms

If a parent/caregiver records in the eCOA or informs the site that the neonate/infant experienced any signs or symptoms suggesting possible COVID-19, this will be considered **COVID-19 Day 1** (day of onset of signs and symptoms). The parent(s)/caregiver(s) will be asked to complete the electronic ObsRO (ie, the PedSIC, including body temperature) in the eCOA.

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[a] long-term sequelae of COVID-19 will not be followed until their resolution

[b] Closure of a COVID-19 episode should occur at the last study visit. If the episode is ongoing, it should be marked as such in the eCRF. The episode will be followed by the investigator until resolution or until a clinically stable condition is reached, and the outcome recorded in the participant's medical chart.

Notes:

The PedSIC questionnaire asks the parent(s)/caregiver(s) if the neonate/infant had any of the prespecified signs or symptoms during the past 24 hours, and (when applicable) to rate the severity. The PedSIC questionnaire takes approximately 5 minutes to complete.

The parent(s)/caregiver(s) should measure body temperature of the neonate/infant (in accordance with the local standard of care) in the evening. If more than one measurement is made, the highest temperature of that day will be recorded in the PedSIC.

The parent(s)/caregiver(s) should record at least 1 of the 3 pulse oximetry readings in the last 24 hours in the eCOA.

If a parent/caregiver is unable to complete the PedSIC in the eCOA, a study staff member can collect information on the neonate/infant's symptoms, body temperature and pulse oximetry, by contacting the caregiver by telephone (or visit the neonate/infant at home, if allowed by local regulations), reading the questions aloud to the caregiver and entering the caregiver's responses on the caregiver's behalf. More details are provided in the eCOA Study Manual.

If a participant is unable to complete the PedSIC in eCOA, the reason for missing the PedSIC completion should be recorded in the eCRF.

Based on the information collected through the PedSIC, the site will reach out to the parent(s)/caregiver(s) at the latest on COVID-19 Day 2 (the day after the day of symptom onset) to assess whether the reported signs and symptoms qualify as a suspected COVID-19 episode using prespecified criteria (Section 8.1.2.5 and 8.1.2.6). If the caregiver would actively reach out to the site already on COVID-19 Day 1, the site should already make a first assessment on COVID-19 Day 1 to check whether the reported signs and symptoms qualify as a suspected COVID-19 episode using prespecified criteria (Sections 8.1.2.5 and 8.1.2.6). As soon as the prespecified criteria for suspected COVID-19 are met (**COVID-19 Day 1-3**), the parent(s)/caregiver(s) of the neonate/infant will be asked to undertake the COVID-19 procedures. In particular:

The parent(s)/caregiver(s) will be asked to continue to complete the ObsROs in the eCOA on behalf of the neonate/infant, as specified above for COVID-19 Day 1:

- PedSIC (including body temperature): every day, preferably in the evening around the same time each day. If more than one measurement is made on any given day, the highest temperature of that day will be recorded.
- Blood oxygen saturation and pulse rate using a pulse oximeter 3 times a day, preferably in the morning, at lunch time, and in the evening.

  Note: the ObsROs do not have to be completed if special circumstances occur, such as hospitalization, in which case the reason for not completing the ObsROs should be recorded by site staff in the eCRF.

A trained HCP will collect a nasal swab from the neonate/infant on **COVID-19 Day 1-3**, as soon as possible after it has been confirmed that the prespecified criteria for suspected COVID-19 are met. In case if the nasal swab is collected at the participant's home, the

study site should arrange transfer of the nasal swab to the study site within 3 days after collection. The COVID-19 Day 1-3 nasal swab can also be collected at the study site by the trained HCP (or hospital or other location, if needed), if preferred by the parent(s)/caregiver(s).

Day 1-3 Procedures in Case of a Positive RT-PCR Test Outside the Study Site Context (Including a Positive RT-PCR Test of a Close Relative from the Same Household)

If a parent/caregiver becomes aware of a positive RT-PCR test for SARS-CoV-2 for the neonate/infant or from a close relative from the neonate/infant, the parent/caregiver should contact the site as soon as possible. The day the parent/caregiver became aware of the positive PCR test will be considered **COVID-19 Day 1**. Regardless of whether the neonate/infant is symptomatic or asymptomatic, the parent/caregiver will be asked to:

Complete the (suspected) COVID-19 surveillance (pediatric symptom check) in the eCOA. In case of COVID-19-like signs and symptoms they will need to complete the PedSIC, including body temperature and pulse oximetry, results in the eCOA.

In case of positive RT-PCR test from mother or close relative from the same household: site staff will collect, regardless of whether there are signs of infection in the infant, a nasal swab from the neonate/infant on **COVID-19 Day 1-3**, as described for the neonate/infant with signs and symptoms (see above). The day the parent/caregiver became aware of the positive PCR test will be considered **COVID-19 Day 1**.

In case of a positive RT-PCR test for the neonate/infant from outside the study: the **COVID-19 Day 4-7** procedures will be initiated, as described below. COVID-19 Day 1-3 is not expected.

If a neonate/infant has a positive test result for SARS-CoV-2 infection, the parent(s)/caregiver(s) will be notified and may be requested to keep the neonate/infant at home and not visit the study site. If necessary, study-site personnel may visit the neonate/infant at home[a] (if allowed by local regulations). The parent(s)/caregiver(s) will be contacted by the site at least once per week to follow-up on the neonate/infant's condition. The PedSIC will be reviewed by study staff during these contacts (phone call or visit).

If a neonate/infant has a negative test result for SARS-CoV-2 infection, the parent(s)/caregiver(s) and neonate/infant will not undertake any further COVID-19 procedures and will fall back to the default Schedule of Activities.

[a] The study staff visiting participants at home will use personal protective equipment according to local regulations.

Day 4-7 Procedures for all Neonates/Infants Who Have met the Prespecified Criteria for (Suspected) COVID-19

The parent(s)/caregiver(s) will be asked to come to the site with the neonate/infant on **COVID-19 Day 4-7** (between 1 and 6 days after symptom onset and becoming aware of a positive RT-PCR test on COVID-19 Day 1-3 or a positive RT-PCR test from outside the study).

If a site visit is not feasible, a member of the study staff could visit the neonate/infant at home (or at the hospital or other location, if needed), if allowed by local regulations. The study staff visiting participants at home will use personal protective equipment according to local regulations. The COVID-19 Day 4-7 assessments may also be performed by a trained HCP, if allowed per local regulations.

For all neonates/infants with confirmed COVID-19 (by molecular diagnostic RT-PCR), study staff will measure vital signs (body temperature, heart rate, and respiratory rate) and oxygen saturation (via pulse oximetry). A targeted physical examination will be performed based on the judgement of the investigator or designated medically qualified healthcare professional.

A nasal swab (only for participants who had a positive RT-PCR test from outside the study) will be collected from the participant by a trained member of the study site staff. In case the nasal swab is taken at home, the study site should arrange transfer of the nasal swab to the study site as soon as possible after collection (within 3 day maximum).

A blood sample will be collected by a study staff and the procedures for the 7-day cycles should be started (see below). The medical history and description of COVID-19 episode will be collected by interview with the parent(s)/caregiver(s).

Procedures During the 7-day Cycles

If a neonate/infant has a positive nasal sample for SARS-CoV-2 at COVID-19 Day 1-3 or has a positive nasal sample for SARS-CoV-2 from outside the study, the parent(s)/caregiver(s) will be asked to undertake the COVID-19 procedures, in particular:

In case of signs and symptoms: The parent(s)/caregiver(s) will be reminded to further complete the ObsROs in the eCOA as described for COVID-19 Day 1-3:

In case of positive PCR and asymptomatic: The parent(s)/caregiver(s) will be reminded to further complete (suspected) COVID-19 surveillance (pediatric symptom check at least twice a week).

In case if the nasal swab is collected at the participant's home, the study site should arrange transfer of the nasal swabs to the study site within 3 days after collection.

Note: Parent(s)/caregivers should be encouraged by the site to allow the collection of nasal swabs from the neonate/infant as indicated in Section 1.3.5. If the parent/caregiver is unable or unwilling to collect all samples as requested, the neonate/infant should still complete the other COVID-19 assessments, including the visit at COVID-19 Day 29.

COVID-19 Day 29 Procedures

If a neonate/infant has a SARS-CoV-2 positive nasal swab collected on COVID-19 Day 1-3 or from outside the study, then the parent(s)/caregiver(s) will be asked to return to the site with the neonate/infant on COVID-19 Day 29 (±7 days) where a blood sample will be drawn from the neonate/infant. Study staff will measure vital signs (body temperature, heart rate, and respiratory rate) and oxygen saturation (via pulse oximetry). A targeted physical examination will be performed based on the judgement of the investigator or designated medically qualified healthcare professional. The medical history and description of COVID-19 episode will be collected by interview with the parent(s)/caregiver(s). If the neonate/infant is still symptomatic, the parent(s)/caregiver(s) will complete the PedSIC in the eCOA. For asymptomatic neonates/infants, the (suspected) COVID-19 surveillance (pediatric symptom check) will be completed by the parent(s)/caregiver(s).

Notes: COVID-19 Day 29 procedures should still be performed even if the nasal swabs results are still pending. The COVID-19 Day 29 assessments may also be performed by a trained HCP at the participant's home, if allowed per local regulations.

This visit can be combined with a regular study visit if within the applicable visit windows.

Closure of the COVID-19 Episode

The parent(s)/caregiver(s) and neonate/infant should continue the COVID-19 procedures until any of the following occurs, based on molecular test results:

If the nasal swab collected from the neonate/infant on COVID-19 Day 1-3 is **negative** for SARS-CoV-2, the parent(s)/caregiver(s) and neonate/infant will not undertake any further COVID-19 procedures and will fall back to the default Schedule of Activities.

For participants with a positive test result for SARS-CoV-2 infection, a study visit will be conducted 28 days after symptom onset (ie, at the COVID-19 Day 29 visit) to assess the clinical course of the infection.

If the neonate/infant has a SARS-CoV-2 positive nasal swab collected on COVID-19 Day 1-3 or from outside the study, then the parent(s)/caregiver(s) will be asked to undertake the COVID-19 procedures with the neonate/infant until 14 days after symptom onset (COVID-19 Day 15, if applicable) or until **resolution of the COVID-19 episode**, whichever comes last[a]. Resolution of the COVID-19 episode is defined as having 1 SARS-CoV-2 negative nasal swab and 2 consecutive days with no COVID-19-related signs or symptoms. Once past COVID-19 Day 15, nasal swabs will no longer be collected from the neonate/infant as soon as 1 nasal swab is SARS-CoV-2 negative, but (if the neonate/infant is still symptomatic at that time) the parent(s)/caregiver(s) should continue completing the ObsROs (including PedSIC, body temperature, and pulse oximetry) in the eCOA until 2 consecutive days with no COVID-19-related signs or symptoms.

[a] long-term sequelae of COVID-19 will not be followed until their resolution

Note: for neonates/infants who have signs and symptoms present at baseline (assessed pre-vaccination), only signs and symptoms that are associated with COVID-19 and that developed during the COVID-19 episode are to be taken into account.

Upon closure of the COVID-19 episode and procedures, all neonates/infants will fall back to the default Schedule of Activities (Section 1.3.3).

All confirmed COVID-19 episodes will be communicated to the respective parent(s)/caregiver(s) if required per local regulations.

If the neonate/infant experiences new signs or symptoms suggesting possible COVID-19 at a later point in time, the neonate/infant would restart the COVID-19 procedures from COVID-19 Day 1 onwards.

8.1.2.3. Procedures for Neonates Born to Mothers With Confirmed COVID-19

Testing for SARS-CoV-2 should be performed for all neonates, regardless of whether there are signs of infection in the neonate, if they were born to mothers with confirmed COVID-19 at the time of birth, or if a close relative from the same household has confirmed COVID-19 at the time of birth.

Testing for diagnosis of SARS-CoV-2 infection will be performed on nasal swab samples by the local laboratory.

Testing of asymptomatic and symptomatic neonates should be performed as soon as feasible. If initial test results are negative, or not available, testing should be repeated for confirmation.

For asymptomatic neonates expected to be discharged at <48 hours of age, a single test can be performed prior to discharge, between 24-48 hours of age (recommendation).

It is recommended, for neonates presenting with signs of infection suggestive of COVID-19 (including fever, lethargy, rhinorrhea, cough, tachypnea, difficulty breathing, vomiting, diarrhea, bluish lips or face, red or bruised looking feet or toes, or poor feeding), or with a positive SARS-CoV-2 test, refer to a hospital as outlined in the procedures in Section 8.1.2.2.

8.1.2.4. Prespecified Criteria for Suspected COVID-19 in Adults

For adults, the criteria for suspected COVID-19 (ie, the triggers to proceed with contacting the study site and home-collection of the nasal swab) are prespecified as follows:

A positive RT-PCR result for SARS-CoV-2, through a private or public laboratory independent of the study, whether symptomatic or asymptomatic

OR

New onset or worsening of any 1 of these symptoms, which lasts for at least 24 hours, not otherwise explained:

- Headache
- Malaise (appetite loss, generally unwell, fatigue, physical weakness)
- Myalgia (muscle pain)
- Chest congestion
- Cough
- Runny nose
- Shortness of breath or difficulty breathing (resting or on exertion)
- Sore throat
- Wheezing
- Eye irritation or discharge
- Chills
- Fever (≥38.0°C or ≥100.4°F)
- Pulse oximetry value ≤95%, which is a decrease from baseline
- Heart rate ≥90 beats/minute at rest, which is an increase from baseline
- Gastrointestinal symptoms (diarrhea, vomiting, nausea, abdominal pain)
- Neurologic symptoms (numbness, difficulty forming or understanding speech)
- Red or bruised looking toes
- Skin rash
- New or changing olfactory or taste disorders
- Symptoms of blood clots: pain/cramping, swelling or redness in your legs/calves
- Confusion
- Bluish lips or face
- Clinical suspicion/judgement by investigator of symptoms suggestive of COVID-19

As several of the prespecified criteria for suspected COVID-19 overlap with vaccine-related reactogenicity, investigators' clinical judgement is required to exclude vaccine-related events when assessing suspected COVID-19.

8.1.2.5. Prespecified Criteria for Suspected COVID-19 for Neonates

For neonates, the criteria for suspected COVID-19 (ie, the triggers to proceed with contacting the study site and collection of the nasal swab by the HCP) are prespecified as follows:

A positive RT-PCR result for SARS-CoV-2 for the neonate, through a private or public laboratory independent of the study, whether symptomatic or asymptomatic

OR

A positive RT-PCR result for SARS-CoV-2 for a parent/caregiver, or any other close relative of the neonate, through a private or public laboratory independent of the study, whether symptomatic or asymptomatic

OR

New onset or worsening of any 1 of these symptoms[a], which lasts for at least 24 hours, not otherwise explained:

- Fever (≥38.0°C or ≥100.4°F)
- Lethargy (decreased activity)
- Nasal congestion of runny nose (rhinorrhea)
- Cough
- Tachypnea (according to age)
- Difficulty breathing
- Vomiting
- Diarrhea
- Poor feeding
- Bluish lips or face
- Bloodshot eyes
- Red or bruised looking feet or toes
- Clinical suspicion/judgement by investigator of symptoms suggestive of COVID-19, including MIS-C

[a] Adapted from Section 10.10.2

8.1.2.6. Prespecified Criteria for Suspected COVID-19 for Infants

For infants, the criteria for suspected COVID-19 (ie, the triggers to proceed with contacting the study site and collection of the nasal swab by the HCP) are prespecified as follows:

A positive RT-PCR result for SARS-CoV-2 for the infant, through a private or public laboratory independent of the study, whether symptomatic or asymptomatic

OR

A positive RT-PCR result for SARS-CoV-2 for a parent/caregiver, or any other close relative of the infant, through a private or public laboratory independent of the study, whether symptomatic or asymptomatic

OR

New onset or worsening of any 1 of these symptoms[a], which lasts for at least 24 hours, not otherwise explained:

- Fever (≥38.0°C or ≥100.4°F) or chills
- Cough
- Wheezing
- Nasal congestion or runny nose (rhinorrhea)
- Shortness of breath or difficulty breathing
- Diarrhea
- Vomiting
- Lethargy/Tiredness/Decreased activity
- Poor appetite or poor feeding
- Mood behaviors (frequent crying, irritability)
- Tachypnea (according to age)
- Rash
- Bloodshot eyes
- Bluish lips or face
- Red or bruised looking feet or toes
- Clinical suspicion/judgement by investigator of symptoms suggestive of COVID-19, including MIS-C

[a] Adapted from Section 10.10.3

8.1.3. Symptomatic SARS-COV-2 Infection

Identification and molecular confirmation of SARS-CoV-2 infection and symptomatic COVID-19 will be performed throughout the study as described in Section 8.1.2. The ePRO/eObsRO to evaluate exploratory vaccine parameters will be the SIC/PedSIC.

Molecular confirmation of SARS-CoV-2 infection by a central laboratory will be used for the exploratory analysis of the case definition only. The severity of all COVID-19 cases will be assessed using the case definitions in Section 10.8.

8.1.4. Asymptomatic SARS-COV-2 Infection

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.

Asymptomatic infections also include those detected by confirmed molecular tests (either in this study or outside study), in the absence of the relevant symptoms.

8.2. Safety Assessments

Details regarding the IDMC are provided in Section 10.3.6.

AEs will be reported and followed by the investigator as specified in Section 8.3 and Section 10.4.

Any clinically relevant changes occurring during the study must be recorded in the eCRF, as specified in Section 8.3.1.

Any clinically significant abnormalities persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable condition is reached.

The study will include the following evaluations of safety and reactogenicity according to the time points provided in the Schedules of Activities (Section 1.3).

8.2.1. Physical Examinations

A full physical examination, including height and body weight, will be carried out at screening.

At all other visits, an abbreviated, symptom-directed examination might be performed by the investigator based on any clinically relevant issues or symptoms, and medical history. Symptom-directed physical examination may be repeated if deemed necessary by the investigator.

Physical examinations will be performed by the investigator or designated medically qualified healthcare professional Any clinically relevant abnormalities or changes in severity observed during the review of body systems should be documented in the eCRF, in accordance with Section 8.3.1.

The following physical examinations will be performed:

Full physical examination includes height, body weight[a], examination of skin, respiratory, cardiovascular, abdominal, limited neurological, musculoskeletal, external ano-genital systems (if clinically necessary), general appearance, head/ear/nose/throat, neck and extremities. This will be carried out at screening, on the day of vaccination (Study Day 1 and the Booster Visit, if applicable). A full physical examination will be performed at the Early Exit visit, if applicable.

Obstetric examination will include measurement of fundal height, fetal heart tones, determination of position of the fetus, and internal manual pelvic examination (if clinically necessary). This will be carried out at screening, on the day of vaccination (Study Day 1 [-14 days and +14 days]). If pregnant at the time of the Early Exit visit, an obstetric examination will be performed.

Obstetric ultrasound is performed by a qualified provider to confirm normal intrauterine pregnancy, estimate gestational age, and identify any fetal abnormalities as appropriate to stage of pregnancy (intrauterine growth retardation, congenital malformations).

Targeted physical examination includes an assessment of any systems that are indicated by history or observation. This will be carried out on Study Day 8 (7 days after vaccine) ± 3 days, Study Day 29 (28 days after the vaccine) ± 7 days, PP1, 6 months, and 12 months postpartum. If applicable, participants who receive the booster vaccination will have a targeted examination at the Day 29 PB visit.

Neonatal physical examination includes gestational age, sex (male, female or indeterminate), (birth) weight, length and head circumference, general appearance (syndromic or normal), generalized dermatological signs, cardiovascular signs, respiratory signs, hematological signs, gastrointestinal signs, urogenital signs, musculoskeletal signs, neurological signs (including audiological test results), neurodevelopmental signs, ocular/visual signs, endocrine/metabolic signs; presence of congenital malformations or birth injuries; presence of signs of congenital or acute infection in the neonate, and the overall Apgar score (Apgar 2015).

The overall Apgar score is required to be documented in the eDC. The Apgar score comprises of the parameters: include color, heart rate, reflexes, muscle tone, and respiration that could be entered in the eDC if available. The assessment is performed at 1 minute and 5 minutes after birth, and may be checked at 10 minutes and 20 minutes after birth, if required (see Neonate/Infants Schedule in Section 1.3.3). Further details of the Apgar score can be found in Section 10.4.8.

Infant physical examination includes weight, length, head circumference, general appearance, skin, head and neck, cardiovascular, neurologic, respiratory, abdominal and musculoskeletal examination.

[a] To obtain the actual body weight, participants must be weighed lightly clothed. The height should be measured without footwear.

8.2.2. Vital Signs

Body temperature (oral route preferred for adults, in accordance with the local standard of care for neonates/infants), pulse/heart rate, respiratory rate, blood pressure (adults only), and blood oxygen saturation will be assessed.

Adult participants will utilize a reactogenicity diary to record body temperature measurements from the time of vaccination until 7 days after vaccination in the eCOA (see Section 8).

All participants with COVID-19 signs and symptoms should measure body temperature daily (in accordance with the local standard of care) and record the highest temperature in the last 24 hours each day in the ePRO in the eCOA, for the duration of follow-up of COVID-19 episodes (as defined in Section 8.1.2.1).

All parent(s)/caregiver(s) of neonates/infants with (suspected) COVID-19 should measure blood oxygen saturation 3 times a day and body temperature daily (the highest temperature in the last 24 hours each day) and record it in the eCOA for the duration of follow-up of COVID-19 episodes (as defined in Section 8.1.2.2).

Blood pressure and pulse/heart rate measurements should be preceded by at least 5 minutes of rest in a quiet setting without distractions (eg, television, cell phones). Vital signs are recommended before blood sampling.

8.2.3. Clinical Safety Laboratory Assessments

Blood samples for hematology and a random urine sample for pregnancy testing and urine dipstick for urinalysis will be collected as noted in Section 10.2. The investigator must review the laboratory results, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF, in accordance with Section 8.3.1.

8.2.3.1. Hematology Clinical Laboratory Assessments

Blood samples for hematology (as detailed in Section 10.2) will be collected for adults at the timepoints indicated in the Schedules of Activities (see Section 1.3).

Serum samples originally drawn or yet to be drawn for the humoral immunogenicity assessments could be used for the testing of relevant coagulation biomarkers

The investigator must review the laboratory results, document this review, and record any clinically relevant changes occurring during the study in the AE or medical history (general and obstetric) section of the CRF, as applicable.

Plasma and serum derived from whole blood samples will be collected from all participants at selected time points for retrospective analysis of hematological parameters, including pro- and anti-coagulation factors, in the event of a suspected AESI of TTS post-vaccination.

Coagulation factors, platelet counts and cell blood count (CBC) will be determined locally. The extent to which levels of these coagulation factors fluctuate pre-and post-vaccination with Ad26.COV2.S may be investigated for research purposes.

Every effort should be made to collect blood samples from the participant for a platelet count (local laboratory or substitute for local laboratory) and other applicable testing (central laboratory) (see the Schedule of Activities in Section 1.3.6 and Section 10.2, Appendix 2).

In case of a post-vaccination thrombotic event or TTS, every effort should be made to collect local hospital/laboratory test results obtained by the treating physician to allow rapid diagnosis and treatment. This information should be reported through the TTS AESI form (see Section 10.11, Appendix 11) electronically per instructions in the eCRF completion guidelines.

See Section 8.3.6.1 for details on laboratory test details to be reported for an AE of thrombocytopenia.

8.2.4. Medical, Obstetric and Delivery History

Medical history includes general medical history including preexisting non-obstetric conditions, previous surgery, medication history up to one month prior to conception/pregnancy, and hospitalizations.

Obstetric medical history includes order of the current pregnancy, gravidity, parity, attendance at antenatal visits and results of any routine tests obtained during pregnancy to assess pregnancy and the fetus (eg, congenital anomalies, ultrasound, amniocentesis). For prior pregnancies, dates of delivery or termination of pregnancy, history of multiple pregnancies, pregnancy complications, history of caesarean section (elective or emergency), pregnancy outcome(s) (live birth, still birth or abortions, c-sections, multiple pregnancies and complications if applicable) and history of previous early-onset neonatal infection, if feasible.

Delivery history includes place of delivery (District Hospital or other); mode of delivery (normal spontaneous vaginal delivery, elective versus emergency caesarean section); length of first stage of labor, length of second stage of labor; date and time of rupture of membranes; date and time of birth; presence and type of health care assistant at delivery (physician, midwife, other); delivery complications (including blood loss and intrapartum infection); singleton vs multiple birth.

8.2.5. Breast Milk Production Outcomes

Subjective changes in (postpartum) breast milk production (reduction) will be collected in the eCOA diary.

8.2.6. Neonatal Assessments

Neonatal medical history includes birth/termination outcome (live birth, stillbirth, termination/miscarriage, neonatal death) demographics, physical examination, overall Apgar score, weight, length, head circumference, and key findings of fetal monitoring during labor such

as fetal presentation at delivery, gestational age (ultrasound), complications, fetal heart rate and uterine contractions during labor.

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). If any infant scores in the "gray" or "black" zones, the participant will be advised to discuss with the infant's pediatrician.

8.3. Adverse Events, Serious Adverse Events, Adverse Events of Special Interest, and Other Safety Reporting

Timely, accurate, and complete reporting and analysis of safety information, including AEs, suspected, SAEs, AESIs, pregnancy-related AEs throughout pregnancy, MAAEs, and product quality complaint (PQC), from clinical studies are crucial for the protection of participants, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established Standard Operating Procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of safety information; all clinical studies conducted by the sponsor, or its affiliates will be conducted in accordance with those procedures.

AEs will be reported by the participant (or, when appropriate, by a parent/caregiver, surrogate, or the participant's legally acceptable representative) for the duration of the study.

Further details on AEs, suspected SAEs, AESI, pregnancy-related AEs throughout pregnancy, MAAEs, and PQC can be found in Section 10.4.

8.3.1. Time Period and Frequency for Collecting Information Relating to Adverse Events, Adverse Events of Special Interest, Serious Adverse Events, Pregnancy-related Adverse Events and Medically-attended Adverse Events

All Adverse Events

All AEs and special reporting situations, whether serious or non-serious, that are related to study procedures or that are related to non-investigational sponsor products will be reported for all participants (adult participants and neonates/infants) from the time a signed and dated ICF is obtained until the end of the study/early withdrawal.

Clinically relevant medical events not meeting the above criteria and occurring between signing of the ICF and moment of first vaccination will be collected on the Medical History eCRF page as pre-existing conditions.

For adult participants, solicited AEs, collected through a reactogenicity diary as part of the eCOA, will be recorded for vaccination from the time of vaccination until 7 days post-vaccination (or until resolution).

All other unsolicited AEs and special reporting situations, whether serious or non-serious, will be reported from the time of vaccination until 28 days post-vaccination. Unsolicited AEs with the onset date outside the timeframe defined above (>28 days after previous study vaccination), which are ongoing on the day of the subsequent vaccination, should be recorded as such.

All SAEs, AESI, AEs and pregnancy-related AEs throughout pregnancy leading to discontinuation from the study/vaccination (regardless of the causal relationship) are to be reported for all adult participants from the moment of first vaccination until completion of the participant's last study-related procedure, which may include contact for safety follow-up. The investigator's assessment of ongoing AEs at the time of each participant's last visit should be documented and closed in the participant's medical record. The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

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. MAAE are to be reported for all participants from the moment of the vaccination until 6 months after vaccination. MAAEs leading to study discontinuation which are to be reported until completion of the participant's last study-related procedure. New onset of chronic diseases will be collected as part of the MAAEs.

AEs leading to discontinuation will be reported throughout the study.

All AEs will be followed until resolution or until clinically stable.

Adverse Events of Special Interest

From the time of local approval of protocol Amendment 3 onwards, post-vaccination TTS is considered to be an AESI. Suspected AESIs (thrombotic events and thrombocytopenia [defined as platelet count below 150,000/µL post-vaccination, see Brighton Collaboration Interim Case Definition, 2021]) 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 and determine whether it is a case of TTS (see Section 8.3.6).

Serious Adverse Events

All SAEs, as well as PQC, occurring during the study must be reported to the appropriate sponsor contact person by study site personnel within 24 hours of their knowledge of the event.

Serious adverse events, including those spontaneously reported to the investigator within 30 days after the last dose of study vaccine, must be reported using an SAE form. The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

SAEs in adult participants will be assessed and reported throughout the study, from the date of vaccination until end of study or at least 12 months postpartum. SAEs in the neonates/infants will be followed up until end of study (up to approximately 12 months of age).

Information regarding SAEs will be transmitted to the sponsor using the Serious Adverse Event Form and Safety Report Form of the eCRF, which must be completed and reviewed by a physician from the study site and transmitted to the sponsor within 24 hours. The initial and follow-up reports of an SAE should be transmitted electronically or by facsimile (fax). Telephone reporting should be the exception and the reporter should be asked to complete the appropriate form(s) first.

Signs and symptoms of Multisystem Inflammatory Syndrome in Children (MIS-C) will be monitored and reported as SAE from birth until study end in neonates/infants with (suspected) COVID-19 as confirmed by a positive RT-PCR result for SARS-CoV-2, and in neonates/infants of mothers or a close relative with prior (within 4-weeks) or current suspected or confirmed COVID-19. Further details on case definition and reporting of MIS-C in infants can be found in Section 10.7.

8.3.2. Method of Detecting Adverse Events, Adverse Events of Special Interest, Serious Adverse Events and Medically-attended Adverse Events

Care will be taken not to introduce bias when detecting AEs, suspected AESI, or SAEs. Open-ended and nonleading verbal questioning of the adult participant is the preferred method to inquire about AE occurrence.

Study site personnel, as well as parent(s)/caregiver(s), should be instructed how to interpret signs and symptoms (eg, crying and pain) in their individual neonate/infant. They will be instructed to report both specific and non-specific symptoms (including vomiting, diarrhea, sleepiness, variation in the intensity and pattern of crying, etc.). These non-specific symptoms may be the only manifestations of some adverse reaction observed in neonates/infants. Care should be taken that the clinical presentation of adverse reactions is not misinterpreted as the manifestation of a preexisting or unrelated condition.

Moreover, symptoms that are dependent on participant communication ability (eg, nausea, pain, mood alterations) in neonates/infants could potentially be at risk for under- or misreporting.

Solicited Adverse Events

Solicited AEs are used to assess the reactogenicity of the study vaccine and are predefined local (at the injection site) and systemic events for which the adult participant is specifically questioned, and which are noted by participants in their reactogenicity diary.

After vaccination, adult participants will remain under observation at the study site for at least 30 minutes (1 hour for Sentinel and Safety Cohorts) for the presence of any severe acute reactions and solicited events.

In addition, adult participants will record solicited signs and symptoms in a reactogenicity diary for 7 days after vaccination. All adult participants will be provided with a reactogenicity diary and instructions on how to complete the reactogenicity diary (see Overview in Section 8). The reactogenicity diary information will be transferred to the sponsor. After review and verbal discussion of the initial reactogenicity diary entries with the participant, the investigator will

complete his/her own assessment in the relevant sections of the eCRF/eCOA. Once a solicited sign or symptom from the reactogenicity diary is considered to be of severity Grade 1 or above, it will be recorded as a solicited AE.

Solicited Injection Site (Local) Adverse Events

Adult participants will be asked to note in the reactogenicity diary, occurrences of injection site pain/tenderness, erythema and swelling at the study vaccine injection site daily for 7 days post-vaccination (day of vaccination and the subsequent 7 days). The extent (largest diameter) of any erythema and swelling should be measured (using the ruler supplied) and recorded daily. The case definitions for solicited injection site events can be found in the references (Gidudu 2012, Kohl 2007).

Solicited Systemic Adverse Events

Adult participants will be instructed on how to record daily temperature using a thermometer provided for home use. Adult participants should record the temperature in the reactogenicity diary in the evening of the day of vaccination, and then daily for the next 7 days approximately at the same time each day. If more than one measurement is made on any given day, the highest temperature of that day will be recorded in the reactogenicity diary and used in the relevant sections of the eCRF/eCOA.

Fever is defined as endogenous elevation of body temperature ≥38.0°C or ≥100.4°F, as recorded in at least 1 measurement (Marcy 2004).

Adult participants will also be instructed on how to note signs and symptoms in the reactogenicity diary on a daily basis for 7 days post-vaccination (day of vaccination and the subsequent 7 days), for the following events: fatigue, headache, nausea, and myalgia.

For Group 4 booster participants changes (reduction) in postpartum breast milk production will be recorded in the breat milk diary daily for 7 days post-vaccination and will be evaluated by the PI.

Unsolicited Adverse Events

Unsolicited AEs are all AEs for which the participant is not specifically questioned in the participant's reactogenicity diary.

Adult participants will record unsolicited signs and symptoms of AEs in a reactogenicity diary for 28 days after vaccination.

For details about AESIs, refer to Section 8.3.6.

Medically-attended Adverse Events

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

8.3.3. Follow-up of Adverse Events, Serious Adverse Events, Adverse Events of Special Interest, Pregnancy-related Adverse Events, and Medically-attended Adverse Events

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and evaluations as medically indicated to elucidate the nature and causality of the AE, SAE, suspected AESI, pregnancy-related AEs throughout pregnancy, MAAE, or PQC as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

AEs, including pregnancy, will be followed by the investigator as specified in Section 10.4.

8.3.4. Regulatory Reporting Requirements for Serious Adverse Events

The sponsor assumes responsibility for appropriate reporting of AEs to the regulatory authorities. The sponsor will also report to the investigator (and the head of the investigational institute where required) all suspected unexpected serious adverse reactions (SUSARs). The investigator (or sponsor where required) must report SUSARs to the appropriate IEC/IRB that approved the protocol unless otherwise required and documented by the IEC/IRB. A SUSAR will be reported to regulatory authorities. Participating investigators and IEC/IRB will receive a SUSAR summary.

8.3.5. Pregnancy

All initial reports of pregnancy in participants, with onset after the PP1 visit, must be reported to the sponsor by the study site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and must be reported using an SAE reporting form. Any participant who becomes pregnant after the PP1 visit will not be eligible to receive the booster vaccination. These participants will remain in the study and will continue to undergo all procedures for surveillance and follow-up of COVID-19, immunogenicity and safety as outlined in the protocol for all participants.

8.3.6. Adverse Events of Special Interest

AESIs are significant AEs that are judged to be of special interest because of clinical importance, known class effects, or based on nonclinical signals. AESIs will be carefully monitored during the study by the sponsor. Participants will be reminded daily within the 30 day time period post-vaccination via eCOA diaries if they have experienced any health concerns (including new onset of symptoms such as shortness of breath, chest pain, leg swelling, persistent abdominal pain, severe or persistent headaches or blurred vision, easy bruising, or tiny blood spots under the skin beyond the site of the injection) related to suspected AESI's post-vaccination, and if so, participants will be advised to contact the study center.

AESIs must be reported to the sponsor within 24 hours of awareness irrespective of seriousness (ie, serious and non-serious AEs) or causality following the procedure described above for SAEs.

Specific requirements for the AESI are described below.

8.3.6.1. Thrombosis with Thrombocytopenia Syndrome (TTS)

As described in Section 2.3.1, Risks Related to Study Participation, TTS has been observed very rarely following vaccination with Ad26.COV2.S and is considered to be an AESI in this study. TTS is a syndrome characterized by a combination of both a thrombotic event and thrombocytopenia.

Because this syndrome is rare and not completely understood, all cases of thrombosis and/or thrombocytopenia will be considered a suspected case of post-vaccination TTS until further adjudication can be performed. An AESI Adjudication Committee with appropriate expertise will be established to evaluate each suspected AESI and determine whether it is a case of post-vaccination TTS. The investigator shall be responsible for reporting any suspected AESI of post-vaccination TTS using the SAE form and the form detailed in Section 10.11, Appendix 11: A suspected TTS case is defined as:

- Thrombotic events: suspected deep vessel venous or arterial thrombotic events as detailed in Section 10.12, Appendix 12 OR,
- Thrombocytopenia, defined as platelet count below 150,000/µL (Brighton Collaboration, Interim Case Definition 2021) post-vaccination

Symptoms, signs, or conditions suggestive of a thrombotic event should be recorded and reported as a suspected AESI even if the final or definitive diagnosis has not yet been determined, and alternative diagnoses have not yet been eliminated or shown to be less likely. Follow-up information and final diagnoses, if applicable, should be submitted to the sponsor as soon as they become available.

In the event of thrombocytopenia post-vaccination, study site personnel should report the absolute value for the platelet count and the reference range for the laboratory test used.

In the event of a thromboembolic event or suspected TTS post-vaccination, serum samples obtained both pre- and pos-vaccination should be retrospectively analyzed by the central laboratory for the presence of coagulation factors; repeat testing may be requested for confirmation upon sponsor discretion.

Suspected AESIs will require enhanced data collection and evaluation (see Section 1.3.6). Every effort should be made to report as much information as possible about the AESI to the sponsor in a reasonable timeframe.

If an event meets the criteria for an SAE (Section 10.4.1), it should be reported using the same process as for other SAEs.

The form detailed in Section 10.11, Appendix 11 is intended as a guide for assessment of the AESIs to facilitate diagnosis and determine treatment options. If the investigator is not the treating physician, every effort should be made to collect the information requested in the form from the treating physician and enter the available information in the eCRF. The sponsor will also attempt

to collect information from any thrombotic event, thrombocytopenia, or TTS reported prior to the current protocol Amendment 3.

8.4. Virology Assessments

Nasal swabs will be used to detect and/or quantify SARS-CoV-2. The presence of SARS-CoV-2 infection in nasal swabs will be assessed by the local laboratory (if feasible) and confirmed by the central laboratory using a PCR-based or other molecular diagnostic test.

Gene sequencing may be performed to detect changes in the S gene and potentially also other parts of the viral genome, if a sample is available.

Nasal swabs collected from adult participant or from neonates and infants (to be collected by qualified HCP only) with COVID-19-like symptoms will also be tested at the central laboratory for the presence of other respiratory pathogens using a broad respiratory pathogens panel (results may be shared with the site).

Adult participants, with stable/well-controlled HIV infection, will be encouraged to have HIV RNA viral load and CD4 cell count assessed at least twice a year and to provide these data for inclusion in the eCRF.

8.5. Genetics and Pharmacogenomics

Blood will be drawn at selected timepoints during the study for additional exploratory immunogenicity assessments, which may include limited genomic research (eg, RNAseq) .

8.6. Biomarkers

Blood will be drawn (PAXgene®) at selected timepoints during the study, as indicated in the Schedule of Activities.

For PAXgene® tubes collected in adult participants and in cord blood (if feasible), biomarker analysis may be performed to explore potentially informative biomarkers related to vaccine immunogenicity.

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

8.7. Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9. STATISTICAL CONSIDERATIONS

Statistical analysis will be performed by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the immunogenicity, safety and pregnancy outcomes data is outlined below. Specific details will be provided in the SAP.

9.1. 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.

9.2. Sample Size Determination

The number of adult participants that will be assessed for safety and reactogenicity of the Ad26.COV2.S vaccine, 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. As of Protocol Amendment 7 approval, participant enrollment in this study will be ceased.

Descriptive analysis will be used to evaluate the following:

- Safety and reactogenicity
- Pregnancy outcomes
- Neonate/infant outcomes
- Immunogenicity of Ad26.COV2.S in adult participants and neonates/infants (including immunity conferred by placental transfer of IgG antibodies).

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.

Table 3: Probability of Observing at Least One Adverse Event Given a True Adverse Event Rate

True Adverse	Probability of Observing at Least One Adverse Event			
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).				

Subset of Participants for Th1/Th2 Assessments

The number of the subset of participants that was planned to be assessed for Th1/Th2 cellular immunogenicity of the Ad26.COV2.S vaccine, in participants from Week-16 of pregnancy through to delivery/termination, was chosen to provide a preliminary assessment of Th1/Th2 cellular immunogenicity in this population.

Table 4 provides the probabilities of observing at least one Th2 event given true Th2 rates.

Table 4: Probability of Observing at Least One Th2 Event Given a True Th2 Event

True Th2 Event Rate	Probability of Observing at Least One Th2 Event in N Participants Given a True Th2 Event
	N=25
0.5%	12%
1.0%	22%
2.5%	47%
5.0%	72%
10.0%	93%
25.0%	100%
50.0%	100%

The observation of 0 events is associated with 95% confidence (2-sided) that the true event rate is below the rates specified in Table 5 for the considered number of participants.

Table 5: Upper Limit of the 95% Two-sided Confidence Interval if no Cases are Observed for Different Sample Sizes

Sample Size	N=25	N=40	N=60	N=100	N=120	N=240
Upper Limit 95% two-sided confidence interval	13.7%	8.8%	6%	3.6%	3%	1.5%

9.2.1. Immunogenicity

Adults

The preliminary assessment of the average immunogenicity in the target population will be estimated with the 240 to be enrolled adult participants in the 2nd or 3rd trimester of pregnancy. No formal statistical hypothesis for immunogenicity will be tested. All immunogenicity analyses will be performed on the PPI-A by group (previous vaccination) and overall. Immunogenicity analyses may also be performed by stage of pregnancy (16-27 weeks, 28-38 weeks), administration of booster (Yes, No), SARS-CoV-2 baseline serostatus (Positive, Negative) at which the vaccine was administered and by vaccine history within each group.

The effect of homologous and heterologous boosters on binding antibodies and neutralizing antibody titers will be evaluated descriptively by group and overall.

One of the primary objectives of the study is to assess the humoral immune response in peripheral blood of adult participants induced by Ad26.COV2.S, administered intramuscularly (IM) as a

1-dose (5×10^{10} vp) schedule during the 2nd and/or 3rd trimester of pregnancy, with antibody levels and serological responses measured at 28 days after vaccination by ELISA (S-ELISA, EU/mL).

A secondary objective of this study is 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, with serological responses (including antibody levels) measured at all blood collection timepoints by group and overall (including at the time of delivery [both peripheral blood and cord blood]), by ELISA (S-ELISA, EU/mL).

A further secondary objective is 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 by group and overall, as measured by VNA titers (antibody GMTs), 28 days after vaccination (ie, on Day 29).

Serological response to vaccination as measured by binding (S-ELISA and/or equivalent assay) and neutralizing (VNA) antibody titers will be assessed to evaluate the humoral immune response in adult participants who receive a booster dose, pre-boost and at selected time points post booster vaccination by group and overall.

Neonates and Infants

No formal statistical hypothesis for immunogenicity will be tested. Immunogenicity analyses will be performed on the PPI-NVN set. Analyses may also be performed by vaccination regimen received by the infant's mother during pregnancy and the gestational age at the time of vaccine administration and/or by SARS-CoV-2 baseline serostatus of the infant's mother. The effect of homologous and heterologous boosters on binding antibodies and neutralizing antibody titers in neonates born to previously vaccinated mothers will be evaluated descriptively by group and overall.

A secondary objective of the study is 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 (ie, in cord blood) and at approximately 2 months and 6 months, of age by group and overall, using ELISA (S-ELISA, EU/mL and GMCs).

A further secondary objective of the study is 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 (ie, in cord blood), as measured by VNA titers (antibody GMTs) by group and overall.

9.2.2. Safety

The primary and secondary objectives of the study are to explore the safety and reactogenicity of Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule, during the 2nd and/or 3rd trimester of pregnancy by group and overall. In addition, safety and reactogenicity of a booster vaccination of Ad26.COV2.S at 5×10^{10} vp, in eligible, consenting vaccine naïve participants at study entry (Group 4), administered IM not earlier than 2 months after completion of the

participant's Ad26.COV2.S vaccination in the study. Safety will be evaluated by group and overall by monitoring:

- For adult participants, AEs including solicited local and systemic AEs for up to 7 days after vaccination, or until resolution, and unsolicited AEs for 28 days after vaccination, SAEs, and AESIs observed following vaccination will be collected for the whole study duration and up to 12 months postpartum, and MAAEs (including new onset of chronic diseases) up to 6 months post-vaccination or up to 12 months if resulting in study discontinuation; AEs leading to discontinuation (entire study); pregnancy outcomes (including, live term birth, live preterm birth, stillbirth, and abortion).
- Pregnancy-related AEs throughout pregnancy 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]).
- Outcomes in neonates and infants (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 from birth until approximately 12 months of age [non-exhaustive]), SAEs (including MIS-C), and AESIs in neonates and infants from birth until end of study (approximately 12 months of age), and MAAEs in neonates/infants from birth until 6 months of age or from birth until discontinuation, if the MAAE is resulting in study discontinuation; AEs leading to discontinuation (entire study).

9.3. Populations for Analysis Sets

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled Adults	All adult participants who sign the ICF
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. In addition, samples obtained from participants after SARS-CoV-2 infection occurring after baseline (if applicable) 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 (for example missed vaccinations in the mothers), or neonates/infants with major protocol deviations that are expected to impact the immunogenicity outcomes
The list of major protocol deviations to be excluded 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.	

9.4. Statistical Analyses

The SAP will be finalized prior to the data base lock (DBL) of the primary analysis, and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints. Due to cease of enrollment and limited sample size, some endpoints might not be summarized and will only be provided in listings. More details can be found in the SAP.

9.4.1. General Considerations

Analysis populations are defined in Section 9.3.

Primary and final analyses are defined in Sections 9.5.1 and 9.5.2.

No formal statistical testing of safety or immunogenicity data is planned. Safety and immunogenicity will be summarized with descriptive statistics by group and overall. Safety and immunogenicity analyses may also be performed by stage of pregnancy (16-27 weeks, 28-38 weeks), administration of booster (Yes, No), SARS-CoV-2 baseline serostatus (Positive, Negative) at which the vaccine was administered and by actual vaccine history within each group.

For adult participants, all safety analyses will be performed on the FAS-A and immunogenicity analyses will be performed on the PPI-A by group and overall.

For neonates/infants, safety analyses will be performed on the FAS-NVN and immunogenicity analyses will be performed on the PPI-NVN set by group and overall.

The number and percentage of participants screened, enrolled, vaccinated, received a booster vaccine and completed follow-up will be summarized by group and overall.

Baseline is considered as the last available assessment before vaccination. For neonates baseline is value at birth.

9.4.2. Primary/Secondary Endpoint(s)

Immunogenicity Endpoints

Descriptive statistics (geometric mean and confidence intervals, or median and interquartile range Q1-Q3, as appropriate) will be calculated by group and overall for immunologic parameters for all available time points for adult participants receiving Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy. Descriptive statistics will also be presented for neonates/infants of adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy by group and overall. Several definitions of serological response will be applied (fold increases in GMC [S-ELISA] or GMT [VNA]). Graphical representations of immunologic parameters will be prepared as applicable. Frequency tabulations will be calculated for discrete (qualitative) immunologic parameters, as applicable.

The impact of baseline characteristics on the humoral responses may be explored graphically or with descriptive statistics by group and overall.

For adult participants, immunogenicity analyses may also be performed by stage of pregnancy at which the vaccine was administered, SARS-CoV-2 baseline serostatus and by actual vaccine history within each group.

For neonates/infants, analyses may also be performed by the gestational age at the time of vaccine administration, and SARS-CoV-2 baseline serostatus of the adult participant.

Safety Endpoints

No formal statistical testing of safety data is planned. Safety data will be analyzed descriptively by group and overall for adult participants (including pregnancy outcomes: live term birth, live preterm birth, stillbirth, and abortion) and their neonates/infants up to approximately 12 months of age (including normal neonate, term neonate with (and without) complications, preterm neonate with (and without) complications, neonatal infection, respiratory distress, congenital anomalies, neonatal death, low birth weight, and small for gestational age) for all adult participants receiving the Ad26.COV2.S vaccine. Safety data from participants vaccine naïve at study entry who receive a booster may be provided as a separate descriptive analysis by group and overall (further details are available in the SAP).

For adult participants, safety analyses may also be performed by stage of pregnancy at which the vaccine was administered and by SARS-CoV-2 baseline serostatus.

Adverse Events

The verbatim terms used in the eCRF by investigators to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported AEs and events-related reactogenicity diary information will be included in the analysis: solicited local at injection site and systemic AEs with onset after vaccination, and unsolicited AEs with onset after vaccination. For each AE, the percentage of participants who experience at least one occurrence of the given event will be summarized by group and overall separately for adult participants (up to at least 12 months postpartum) and their neonates/infants (up to approximately 12 months of age).

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]) will be assessed and analyzed for adult participants by group and overall. The complete list of preferred terms to be included as pregnancy-related AEs will be provided in the SAP.

For SAEs, suspected AESIs and AEs leading to discontinuation throughout the study (from first vaccination until end of the study [at least 12 postpartum]), the percentage of adult participants who experience at least 1 occurrence of the given event will be summarized by group, overall and by phase including post booster (if applicable). For MAAEs (from vaccination until 6 months after

vaccination or until end of study when leading to study discontinuation), the percentage of adult participants who experience at least 1 occurrence of the given event will be summarized by group and overall. SAEs in the neonates/infants from birth until end of study (approximately 12 months of age) will be assessed by group and overall. MAAEs in the neonates/infants (from birth until 6 months of age or from birth until discontinuation [if MAAE is leading to study discontinuation]) will be assessed by group and overall.

Summaries, listings, datasets, or participant narratives may be provided, as appropriate, for those participants who die, who discontinue vaccine due to an AE, or who experience a severe AE, an SAE, or a suspected AESI by group and overall.

Solicited local (at injection site) and systemic AEs will be summarized descriptively by group and overall. The number and percentages of participants with at least one solicited local (at injection site) or systemic AE will be presented by group and overall. The frequencies by group and overall as well as frequencies according to severity and duration will be described for solicited AEs . Frequencies of unsolicited AEs, separately for all and vaccination-related only, will be presented by System Organ Class and preferred term, while those of solicited AEs will be presented only by preferred term.

Outcomes

Adverse maternal/fetal outcomes and adverse neonatal/infant outcomes will be defined using the Case Definitions for Pregnancy Outcomes for each endpoint.

The frequency and percentage of participants for whom at least one of the indicated adverse outcomes was reported will be summarized by group and overall. For each separate adverse maternal/fetal and adverse neonatal/infant outcome, the number and percentage of participants who experience at least 1 occurrence of the given event will also be tabulated by group and overall.

9.4.3. Exploratory Endpoint(s)

Transfer of maternal humoral immunity to the infant will be assessed, including:

- levels of maternal IgA and/or other Ig subtypes against SARS-CoV-2 in colostrum and breast milk within 1 week postpartum and at 6 weeks postpartum (Day 43 PP follow-up visit), respectively, if feasible, in a subset of participants who had received Ad26.COV2.S vaccine during the 2nd and/or 3rd trimester of pregnancy. Levels of maternal IgA and/or other Ig subtypes against SARS-CoV-2 will also be assessed in colostrum and/or breast milk, if feasible, at the time of booster vaccination and 28 days post booster vaccination, in participants who were vaccine naïve at study entry and receive a booster after pregnancy completion.
- levels of antibodies in peripheral blood from adult participants and cord blood at the time of delivery. Serological response as measured ELISA will be assessed using GMCs. The GMR (ratio of immunologic parameters between blood and cord blood at time of delivery) will be calculated together with confidence intervals. Additional characterizations will be described in the SAP.

Cellular immune responses induced in participants during the 2nd and/or 3rd trimester of pregnancy after vaccination with Ad26.COV2.S may be further explored using PBMCs obtained on Day 1 (pre-dose), 28 days after vaccination, and at the time of delivery (if feasible), for a subset of participants. With only 1 participant in this subset at the time of writing this Protocol Amendment 7, PBMC sampling may be removed from the study as of Protocol Amendment 7 approval. Analysis will be described in more detail in the SAP.

The correlation between the binding antibody (ELISA) titers and neutralizing antibody (VNA) titers to SARS-CoV-2 will be assessed at selected timepoints using Spearman's rank correlation. Additional details will be provided in the SAP.

The occurrence and severity of COVID-19 infection (asymptomatic and symptomatic) in adult participants or in neonates/infants of participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy, will be assessed using the case definitions in outlined in Section 10.8.

Plasma and serum derived from whole blood samples will be collected from all participants for retrospective analysis of hematological parameters, including pro- and anti-coagulation factors in the event of a suspected AESI of post-vaccination TTS. The extent to which these coagulation factors correlate with platelet counts assessed at the time, as part of the routine CBC assessment will be determined, and the extent to which levels of these coagulation factors fluctuate pre-and post-vaccination with Ad26.COV2.S may be investigated for research purposes. The platelet count will be performed by a local laboratory or substitute for local laboratory. Blood samples will be collected as detailed in Section 1.3 Schedules of Activities.

Developmental outcomes on the ASQ-3 at 2, 6 and 12 months will be summarized.

9.4.4. Other Safety Analyses

Clinical Laboratory Tests

Laboratory data (adult only) will be summarized by group and overall by type of laboratory test. Reference ranges and markedly abnormal results (specified in the SAP) will be used in the summary of laboratory data. Descriptive statistics will be calculated for all laboratory analyte at each time point. A listing of participants with any laboratory results outside the reference ranges will be provided. A listing of participants with any markedly abnormal laboratory results will also be provided.

Vital Signs

Vital signs including body temperature, pulse/heart rate, respiratory rate, and blood pressure (adults only: systolic and diastolic) and blood oxygen saturation will be summarized over time by group and overall, using descriptive statistics and/or graphically. The percentage of participants with values beyond clinically important limits (specified in the SAP) will be summarized by group and overall.

Physical Examinations

Physical examination findings will be summarized at baseline (adults) and at each timepoint for neonates/infants by group and overall. A listing of abnormalities will be made for each scheduled time point.

9.5. Planned Analyses

The SAP will describe the planned primary analysis and final analysis, in greater detail. The results of any analysis may be used for regulatory submissions.

Unplanned interim analyses may be as required or if requested by health authorities.

The IDMC will review safety and reactogenicity data as described in Section 10.3.6.

9.5.1. 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 (AEs, SAEs, and pregnancy outcomes) and immunogenicity data (S-ELISA and VNA) 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.

9.5.2. 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 in Section 1.3. Analysis will include safety (AEs, SAEs, and pregnancy outcomes) and immunogenicity data. Booster dose effect will be evaluated on safety and immunogenicity.

The SAP will describe the planned analyses in greater detail.

Note: Interim analyses may be performed as required or if requested by health authorities.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Abbreviations

Ad26	adenovirus type 26
AdVAC®	adenoviral vaccine (vector platform)
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
Anti-PF4	anti-platelet factor 4
AST	aspartate aminotransferase
BMI	body mass index
BUN	Blood Urea Nitrogen
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention
CLS	Capillary leak syndrome
COPD	chronic obstructive pulmonary disease
COVID-19	coronavirus disease-2019
CPK	creatine phosphokinase
eCRF	Electronic case report form(s)
DBL	data base lock
DNA	deoxyribonucleic acid
DTaP	Diphtheria toxoid, Tetanus toxoid, and acellular Pertussis
DVT	deep vein thrombosis
eCOA	electronic clinical outcome assessment
eDC	electronic data capture
ePRO	electronic patient-reported outcomes
EF-PPND	embryofetal and pre-and postnatal development toxicity
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
ERD	enhanced respiratory disease
FAS	Full Analysis Set
Fc	crystallizable fragment
FDA	Food and Drug Administration
FI	formalin-inactivated
GCP	good clinical practice
GD	gestation day
GLP	good laboratory practice
GMC	Geometric mean concentration
GMT	Geometric mean titer
HCP	health care professional
HELLP	hemolysis elevated liver enzymes and low platelets
HIT	heparin-induced thrombocytopenia
HIV	human immunodeficiency virus
IB	investigator's brochure
ICF	informed consent form
ICS	intracellular cytokine staining
ICH	International Conference on Harmonization
ICU	intensive care unit
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
IM	intramuscular
IPPI	Investigational Product Preparation Instructions
IRB	Institutional Review Board
IWRS	interactive web response system

MAAE	Medically-attended adverse event
MedDRA	Medical Dictionary for Regulatory Activities
MERS (-CoV)	Middle East respiratory syndrome (coronavirus)
MIS-C	Multisystem Inflammatory Syndrome in Children
N	nucleocapsid
ObsRO	Observer-reported outcomes
PBMC	peripheral blood mononuclear cell
PedSIC	Pediatric Symptoms of Infection with Coronavirus-19
PI	principal investigator
PPI	per protocol immunogenicity
PQC	product quality complaint
RNA	ribonucleic acid
RSV	respiratory syncytial virus
RT-PCR	real-time reverse-transcriptase polymerase chain reaction
S	spike
SAE	serious adverse event
SAP	statistical analysis plan
SARS	severe acute respiratory syndrome
SARS-CoV(-2)	severe acute respiratory syndrome coronavirus(-2)
SIC	Symptoms of Infection with Coronavirus-19
SIPPM	site investigational product and procedures manual
SpO_2	oxygen saturation
SRP/S	study responsible physician/scientist
SUSAR	suspected unexpected serious adverse reaction
TTS	Thrombosis with thrombocytopenia syndrome
Th	T helper
Th1/Th2	T helper cell subset(s)
TNF-α	tumor necrosis factor alpha
ULN	upper limit of the normal range
US	United States
VAERD	vaccine-associated enhanced respiratory disease
VSD	Vaccine Safety Datalink
VNA	virus neutralization assay
vp	virus particle
WBC	white blood cell
WHO	World Health Organization

Definition of Terms

COVID-19	COVID-19 is the disease caused by the virus SARS-CoV-2. COVID-19 refers to SARS-CoV-2 infection with symptoms, and can range from mild to severe disease, the latter including pneumonia, severe acute respiratory syndrome, multiorgan failure, and death (US FDA May 2020, US FDA June 2020).
eCOA	Electronic Clinical Outcome Assessment. An umbrella term encompassing different types of outcomes assessments, which may include the COVID-19 signs and symptoms surveillance question, SIC (as an ePRO for adults), PedSIC (as an ObsRO for neonates/infants) and the reactogenicity diary (to record vaccine reactogenicity) in adults. *Note: should the eCOA not be available, paper format will be used to collect the data.*
ePRO	The electronic technology used to collect the patient-reported outcome data. PROs are reports that come directly from the participant without interpretation by clinician or anyone else. This includes the SIC questionnaire and the recording of pulse oximetry results.
reactogenicity diary	Used to record solicited signs and symptoms by the participants.

Electronic source system	Contains data traditionally maintained in a hospital or clinic record to document medical care or data recorded in a CRF as determined by the protocol. Data in this system may be considered source documentation.
ObsROs	Observer-reported Outcomes. Used to collect the observer-reported outcome data. ObsROs are reports that come directly from the legal guardian/caregiver without interpretation by a clinician or anyone else. This includes the PedSIC questionnaire and the recording of pulse oximetry results.

10.2. Appendix 2: Clinical Laboratory Tests

10.2.1. Protocol Required Safety Laboratory Assessments - Adult Participants

The following Hematology, Chemistry and Urinalysis tests will be performed locally within 72 hours of blood sample collection, according to the Schedules of Activities (Section 1.3).

Table 6: Protocol Required Safety Laboratory Assessments to be Performed Locally

Laboratory Assessments	Parameters		
Hematology	Platelet count Hemoglobin	Prothrombin time Activated partial thromboplastin time	White Blood Cell (WBC) count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils
	Note: A WBC evaluation may include any abnormal cells, which will then be reported by the laboratory. In addition, any other abnormal cells in a blood smear will also be reported.		
Clinical Chemistry	Sodium Potassium Blood urea nitrogen (BUN) Creatinine Aspartate aminotransferase (AST) Alanine aminotransferase (ALT)		
Routine Urinalysis	Dipstick* Glucose Protein Blood	Sediment (if dipstick result is abnormal)	
	If dipstick result is abnormal, microscopy will be used to measure sediment. *Urine dipstick analyses will be performed locally and will be confirmed by the central laboratory if the local result is abnormal.		

Table 7: Additional Laboratory Assessments to be Performed as Required

Laboratory Assessments	Parameters	Timepoints
Testing done locally		
Standard Laboratory Assessments	• Urine pregnancy testing	Pre-vaccination at the Booster Vaccination Visit
Testing done centrally		
Hematology Clinical Laboratory Assessments of Coagulation-related parameters (Section 8.2.3.1)	Participants with a suspected AESI: • Serum/plasma samples for coagulation-related assays such as but not limited to: o Activated partial thromboplastin time o Prothrombin time	Based on the clinical evaluation of the suspected AESI (eg, whether thrombocytopenia is observed in conjunction with a thrombotic event), all or some of these tests may be conducted on samples obtained as part of the AESI investigation. Similar samples from appropriate controls (from vaccinated participants who did not experience an

Laboratory Assessments	Parameters	Timepoints
	○ International normalized ratio ○ Fibrinogen ○ D-dimer·levels ○ Lupus anticoagulant· ○ Anti-cardiolipin antibody ○ Beta-2 glycoprotein· ○ HIT/anti-PF4 Antibodies, IgG·(HIT assay) ○ Platelet activation assay (if HIT/anti-PF4 antibodies is positive) ○ Homocysteine· • ADAMTS13 Activity and Inhibitor Profile	AESI) within the study may be used as part of investigation of AESIs. • All or some of these tests may be conducted to assess hematology laboratory parameters in a subset of participants, and the extent to which levels of these factors in peripheral blood fluctuate pre-and post-vaccination with Ad26.COV2.S.

Laboratory abnormalities in adult participants are listed in Section 10.5.

10.3. Appendix 3: Regulatory, Ethical, and Study Oversight Considerations

10.3.1. Regulatory and Ethical Considerations

Investigator Responsibilities

The investigator is responsible for ensuring that the study is performed in accordance with the protocol, current International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of study participants are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the study data are credible.

Protocol Amendments

Neither the investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor, and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the participants, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the investigator and IEC/IRB must be provided to the sponsor. When the change(s) involve only logistic or administrative aspects of the study, the IEC/IRB (where required) only needs to be notified.

During the course of the study, in situations where a deviation from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor representative listed in the Contact Information page(s), which will be provided as a separate document. Except in emergency situations, this contact should be made before implementing any deviation from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the eCRF and source documents will reflect any deviation from the protocol, and the source documents will describe this deviation and the circumstances requiring it.

Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

Required Prestudy Documentation

The following documents must be provided to the sponsor before shipment of study vaccine to the study site:

- Protocol and amendment(s), if any, signed and dated by the PI
- A copy of the dated and signed (or sealed, where appropriate per local regulations), written IEC/IRB approval of the protocol, amendments, ICF, any recruiting materials, and if applicable, participant compensation programs. This approval must clearly identify the specific protocol by title and number and must be signed (or sealed, where appropriate per local regulations) by the chairman or authorized designee
- Name and address of the IEC/IRB, including a current list of the IEC/IRB members and their function, with a statement that it is organized and operates according to GCP and the applicable laws and regulations. If accompanied by a letter of explanation, or equivalent, from the IEC/IRB, a general statement may be substituted for this list. If an investigator or a member of the study site personnel is a member of the IEC/IRB, documentation must be obtained to state that this person did not participate in the deliberations or in the vote/opinion of the study
- Regulatory authority approval or notification, if applicable
- Signed and dated statement of investigator (eg, Form FDA 1572), if applicable
- Documentation of investigator qualifications (eg, curriculum vitae)
- Completed investigator financial disclosure form from the PI, where required
- Signed and dated Clinical Trial Agreement, which includes the financial agreement
- Any other documentation required by local regulations

The following documents must be provided to the sponsor before enrollment of the first participant:

- Completed investigator financial disclosure forms from all subinvestigators
- Documentation of subinvestigator qualifications (eg, curriculum vitae)
- Name and address of any local laboratory conducting tests for the study, and a dated copy of current laboratory normal ranges for these tests, if applicable
- Local laboratory documentation demonstrating competence and test reliability (eg, accreditation/license), if applicable

Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator (or sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents (as required by local regulations):

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any other written materials to be provided to the participants)
- IB (or equivalent information) and amendments/addenda
- Sponsor-approved participant recruiting materials
- Information on compensation for study-related injuries or payment to participants for participation in the study, if applicable

- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for participants
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any, excluding the ones that are purely administrative, with no consequences for participants, data or study conduct, unless required locally), the ICF, applicable recruiting materials, and participant compensation programs, and the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study the investigator (or sponsor where required) will send the following documents and updates to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments (excluding the ones that are purely administrative, with no consequences for participants, data or study conduct)
- Revision(s) to ICF and any other written materials to be provided to participants
- If applicable, new or revised participant recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to participants for participation in the study, if applicable
- New edition(s) of the IB and amendments/addenda
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually)
- Reports of AEs that are serious, unlisted/unexpected, and associated with the study vaccine
- New information that may adversely affect the safety of the participants or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the participants
- Report of deaths of participants under the investigator's care
- Notification if a new investigator is responsible for the study at the site
- Development Safety Update Report and Line Listings, where applicable
- Any other requirements of the IEC/IRB

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for participants, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).

At least once a year, the IEC/IRB will be asked to review and reapprove this study, where required.

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

Country Selection

This study will only be conducted in those countries where the intent is to launch or otherwise help ensure access to the developed product if the need for the product persists, unless explicitly addressed as a specific ethical consideration in Section 4.1.1.

Other Ethical Considerations

For study-specific ethical design considerations, refer to Section 4.1.1.

10.3.2. Financial Disclosure

Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information in accordance with local regulations to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Refer to Required Prestudy Documentation (above) for details on financial disclosure.

10.3.3. Informed Consent Process

Each participant must give written consent according to local requirements after the nature of the study has been fully explained. The ICF(s) must be signed before performance of any study-related activity. The ICF(s) that is/are used must be approved by both the sponsor and by the reviewing IEC/IRB and be in a language that the participant can read and understand. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before enrollment in the study, the investigator or an authorized member of the study site personnel must explain to potential participants the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Participants will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the participant will receive. Finally, they will be told that the investigator will maintain a participant identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorized sponsor personnel without violating the confidentiality of the participant, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the participant is authorizing such access. It also denotes that the participant agrees to allow his or her study physician to recontact the participant for the purpose of obtaining consent for additional safety evaluations, if needed.

The participant will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded

by means of the participant's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the participant.

Informed consent for follow-up of the neonates/infants up to approximately 12 months will be obtained from the parent(s), of which the mother is also an adult participant in this study, at the time of screening of adult participants.

Participants who are rescreened are required to sign a new ICF.

Eligible participants that want to receive the booster vaccination will need to reconsent before any procedure of the Booster Vaccination Visit is performed.

10.3.4. Data Protection

Privacy of Personal Data

The collection and processing of personal data from participants enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of participants confidential.

The informed consent obtained from the participant includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The participant has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

Exploratory research is not conducted under standards appropriate for the return of data to participants. In addition, the sponsor cannot make decisions as to the significance of any findings resulting from exploratory research. Therefore, exploratory research data will not be returned to participants or investigators, unless required by law or local regulations. Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.

10.3.5. Long-Term Retention of Samples for Additional Future Research

Samples collected in this study may be stored for up to 15 years (or according to local regulations) for additional research. Samples will only be used to understand vaccination with Ad26 based vaccines including Ad26.COV2.S, to understand SARS-CoV-2 infection, to understand differential vaccine responders, and to develop tests/assays related to Ad26-based vaccines and to Ad26.COV2.S and SARS-CoV-2 infection. If any trends are observed in thromboembolic events, plasma samples may be tested for markers of thromboembolic events. The research may begin at any time during the study or the post-study storage period.

Stored samples will be coded throughout the sample storage and analysis process and will not be labeled with personal identifiers. Participants may withdraw their consent for their samples to be stored for research (refer to Section 7.3.1).

10.3.6. Safety Monitoring Committees Structure

Independent Data Monitoring Committee

The IDMC will be established to monitor safety data on an ongoing basis to ensure the continuing safety of the participants enrolled in this study. The IDMC consists of members that are not directly involved in the study conduct, data management, or statistical analysis, will be established and will monitor data to ensure the continuing safety of the participants enrolled in this study. The IDMC will consist of at least 1 medical expert in the relevant therapeutic area of pediatrics and at least 1 external statistician independent of the sponsor and not involved in the final analysis of the study who will prepare the data and perform all IDMC safety analyses and interim analyses for review by the IDMC. Committee membership responsibilities, authorities, and procedures will be documented in its charter.

The IDMC will review data as indicated in Section 4.1. IDMC safety reviews will be based on descriptive safety tables and listings from all accumulated safety data at that point in time. After each review, the IDMC will make recommendations regarding the continuation of the study. When appropriate, the conclusions of the IDMC will be communicated to the investigators, the IEC/IRB, and the national regulatory authorities.

The IDMC responsibilities, authorities, and procedures will be provided in its charter.

An ad hoc review may be performed further to the occurrence of any AE/SAE leading to a study pausing situation as outlined in Section 6.9 or at request of the sponsor’s medical monitor or designee. The PI and sponsor's SRP will inform the IDMC of any AE of concern.

It will also be possible for the IDMC to review immunogenicity data during the course of the study if this is deemed necessary for future vaccine development-related decisions. If this is the case, a biomarker representative (not involved in the conduct of the study) will be part of the IDMC.

If any pausing rule is met (refer to Section 6.9) and, if following appropriate safety review it is deemed appropriate to restart dosing, the sponsor must submit a request to restart dosing with pertinent data to competent authority as a request for a substantial amendment, as required by local

regulations or authority request (eg, MHRA). If needed, this will be followed by a substantial amendment of the IB and/or protocol.

10.3.7. Publication Policy/Dissemination of Clinical Study Data

All information, including but not limited to information regarding Ad26.COV2.S or the sponsor's operations (eg, patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the sponsor to the investigator and not previously published, and any data, including exploratory biomarker research data, generated as a result of this study, are considered confidential and remain the sole property of the sponsor. The investigator agrees to maintain this information in confidence and use this information only to accomplish this study and will not use it for other purposes without the sponsor's prior written consent.

The investigator understands that the information developed in the study will be used by the sponsor in connection with the continued development of Ad26.COV2.S, and thus may be disclosed as required to other clinical investigators or regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated by the sponsor and will contain data from all study sites that participated in the study per protocol. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating investigator for the study. Results of exploratory biomarker analyses performed after the Clinical Study Report has been issued will be reported in a separate report and will not require a revision of the Clinical Study Report.

Study participant identifiers will not be used in publication of results. Any work created in connection with performance of the study and contained in the data that can benefit from copyright protection (except any publication by the investigator as provided for below) shall be the property of the sponsor as author and owner of copyright in such work.

Consistent with Good Publication Practices and International Committee of Medical Journal Editors (ICMJE) guidelines, the sponsor shall have the right to publish such primary (multicenter) data and information without approval from the investigator. The investigator has the right to publish study site-specific data after the primary data are published. If an investigator wishes to publish information from the study, a copy of the manuscript must be provided to the sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the sponsor in writing, the investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the sponsor will review these issues with the investigator. The sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multicenter study designs and sub-study approaches, secondary results generally should not be published before the primary endpoints of a study have been published. Similarly, investigators

will recognize the integrity of a multicenter study by not submitting for publication data derived from the individual study site until the combined results from the completed study have been submitted for publication, within 18 months after the study end date, or the sponsor confirms there will be no multicenter study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the ICMJE Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals, which state that the named authors must have made a significant contribution to the conception or design of the work; or the acquisition, analysis, or interpretation of the data for the work; and drafted the work or revised it critically for important intellectual content; and given final approval of the version to be published; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Registration of Clinical Studies and Disclosure of Results

The sponsor will register and disclose the existence of, and the results of clinical studies as required by law. The disclosure of the final study results will be performed after the end of study in order to ensure the statistical analyses are relevant.

10.3.8. Data Quality Assurance

Data Quality Assurance/Quality Control

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and study site personnel before the study, and periodic monitoring visits by the sponsor. Written instructions will be provided for collection, handling, storage, and shipment of samples.

Guidelines for eCRF completion will be provided and reviewed with study site personnel before the start of the study.

The sponsor will review eCRF for accuracy and completeness during on-site monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. After upload of the data into the study database they will be verified for accuracy and consistency with the data sources.

10.3.9. Case Report Form Completion

Case report forms are prepared and provided by the sponsor for each participant in electronic format. All data relating to the study must be recorded in eCRF or eCOA. All eCRF entries, corrections, and alterations must be made by the investigator or authorized study site personnel. The investigator must verify that all data entries in the eCRF are accurate and correct.

The study data will be transcribed by study site personnel from the source documents onto an eCRF, if applicable. Study-specific data will be transmitted in a secure manner to the sponsor.

Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheets will become part of the participant's source documents. Data must be entered into eCRF in English. The eCRF must be completed as soon as possible after a participant visit and the forms should be available for review at the next scheduled monitoring visit.

All participative measurements (eg, questionnaires) will be completed by the same individual who made the initial baseline determinations whenever possible.

If necessary, queries will be generated in the electronic data capture (eDC) tool. If corrections to an eCRF are needed after the initial entry into the eCRF, this can be done in either of the following ways:

- Investigator and study site personnel can make corrections in the eDC tool at their own initiative or as a response to an auto query (generated by the eDC tool).
- Sponsor or sponsor delegate can generate a query for resolution by the investigator and study site personnel.

10.3.10. Source Documents

At a minimum, source documents consistent in the type and level of detail with that commonly recorded at the study site as a basis for standard medical care must be available for the following: participant identification, eligibility (including relevant medical, obstetric and delivery history, and including anything related to the footnotes related to relevant medical and obstetric history, pre-pregnancy and prestudy therapies to the Schedule of Activities [Section 1.3.1 footnote h and Section 1.3.2, footnote h]), and study identification; study discussion and date of signed informed consent; dates of visits; results of safety parameters as required by the protocol; record of all AEs and follow-up of AEs; concomitant medication; vaccine receipt/dispensing/return records; study vaccine administration information; and date of study completion and reason for early discontinuation of study vaccination or withdrawal from the study, if applicable.

The author of an entry in the source documents should be identifiable. Given that ObsROs are reports of an infant participant's health that come directly from the parent(s)/caregiver(s) without interpretation by a clinician or anyone else, the responses to ObsRO measures entered by the parent(s)/caregiver(s) into source records cannot be overridden by site staff or investigators.

Participant/parent(s)/caregiver(s)- and investigator-completed scales and assessments designated by the sponsor (ie, reactogenicity diary to record solicited AEs, a signs and symptoms surveillance question, and SIC/PedSIC) will be recorded and will be considered source data. The participant’s reactogenicity diary used to collect information regarding solicited signs and symptoms after vaccination will be considered source data.

Specific details required as source data for the study and source data collection methods will be reviewed with the investigator before the study and will be described in the monitoring guidelines (or other equivalent document).

An eSource system may be utilized, which contains data traditionally maintained in a hospital or clinic record to document medical care (eg, electronic source documents) as well as the clinical study-specific data fields as determined by the protocol. These data are electronically extracted for use by the sponsor. If eSource is utilized, references made to the eCRF in the protocol include the eSource system but information collected through eSource may not be limited to that found in the eCRF.

10.3.11. Monitoring

The sponsor will use a combination of monitoring techniques central, remote, or on-site monitoring to monitor this study.

The sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study site visit log that will be kept at the study site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the eCRF with the source documents (eg, hospital/clinic/physician's office medical records). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the sponsor and study site personnel and are accessible for verification by the sponsor study site contact. If electronic records are maintained at the study site, the method of verification must be discussed with the study site personnel.

Direct access to source documents (medical records) must be allowed for the purpose of verifying that the recorded data are consistent with the original source data. Findings from this review will be discussed with the study site personnel. The sponsor expects that, during monitoring visits, the relevant study site personnel will be available, the source documents will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

In addition to on-site monitoring visits, remote contacts can occur. It is expected that during these remote contacts, study site personnel will be available to provide an update on the progress of the study at the site.

Central monitoring will take place for data identified by the sponsor as requiring central review.

10.3.12. On-Site Audits

Representatives of the sponsor's clinical quality assurance department may visit the study site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. Remote auditing techniques may also be utilized, if necessary. These audits will require access to all study records, including source documents, for inspection. Participant privacy must, however, be respected. The investigator and study site personnel are responsible for being present and available for consultation during routinely scheduled study site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if he or she has been contacted by a regulatory agency concerning an upcoming inspection.

10.3.13. Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all eCRF and all source documents that support the data collected from each participant, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator/institution must permit access to such reports.

10.3.14. Study and Site Start and Closure

First Act of Recruitment

The first site open is considered the first act of recruitment and it becomes the study start date.

Study/Site Termination

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study vaccine development

10.4. Appendix 4: Adverse Events, Serious Adverse Events, Adverse Events of Special Interest, Product Quality Complaints, and Other Safety Reporting: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.4.1. Adverse Event Definitions and Classifications

Adverse Event

An AE is any untoward medical occurrence in a clinical study participant administered a pharmaceutical (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the vaccine. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per ICH).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Any respiratory tract infection that is not due to SARS-CoV-2 infection will be reported as an AE if it occurs between the time of any vaccination through the following 28 days. Any respiratory tract infection recorded as an AE in the eCRF will be excluded from any AE analysis if the molecular test is subsequently found to be positive for SARS-CoV-2. Respiratory tract infections arising from SARS-CoV-2 infection will not be reported as (S)AEs in the Clinical Study Report but will be tabulated separately. In general, (S)AEs caused by molecularly confirmed SARS-CoV-2 infection will be removed at the analysis level from the (S)AE listings and tables and presented separately.

Note: For time period of sponsor's AE collection, see All Adverse Events under Section 8.3.1.

Serious Adverse Event

An SAE based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (The participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is Medically Important*

*Medical and scientific judgement should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

If a serious and unexpected AE occurs for which there is evidence suggesting a causal relationship between the study vaccine and the event (eg, death from anaphylaxis), the event must be reported as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (eg, all-cause mortality).

If a participant receives a positive SARS-CoV-2 result from a private/off-study test during the study, and the positive result occurs within 28 days after vaccination, the event will be reported as an AE. If it occurs after 28 days, the event will be recorded as an SAE only if the event qualifies as serious. The participant can continue in the study if they choose to; however, this must be in accordance with local country and site level recommendations for COVID-19.

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For Ad26.COV2.S, the expectedness of an AE will be determined by whether or not it is listed in the IB.

10.4.2. Attribution Definitions

Assessment of Causality

The causal relationship to study vaccine is determined by the Investigator. The following selection should be used to assess all AEs.

Related

There is a reasonable causal relationship between study vaccine administration and the AE.

Not Related

There is not a reasonable causal relationship between study vaccine administration and the AE.

The term "reasonable causal relationship" means there is evidence to support a causal relationship.

By definition, all solicited AEs at the injection site (local) will be considered related to the study vaccine administration.

10.4.3. 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 HHS 2007), included in Section 10.5.

For AEs not identified in the grading table, the following guidelines will be applied:

Grade 1	Mild	Symptoms causing no or minimal interference with usual social and functional activities
Grade 2	Moderate	Symptoms causing greater than minimal interference with usual social and functional activities
Grade 3	Severe	Symptoms causing inability to perform usual social and functional activities and requires medical intervention
Grade 4	Potentially life-threatening	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability OR ER visit or hospitalization

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 in Section 10.5. (Note: severity of the measured events will be derived from the diameter [for erythema and swelling] and the temperature measurements [for fever]).

10.4.4. Special Reporting Situations

Safety events of interest on a sponsor study vaccine in an interventional study that may require expedited reporting or safety evaluation include, but are not limited to:

- Overdose of a sponsor study vaccine
- Suspected abuse/misuse of a sponsor study vaccine
- Accidental or occupational exposure to a sponsor study vaccine
- Medication error, intercepted medication error, or potential medication error involving a Johnson & Johnson medicinal product (with or without patient exposure to the Johnson & Johnson medicinal product, eg, product name confusion, product label confusion, intercepted prescribing or dispensing errors)

Special reporting situations should be recorded in the eCRF. Any special reporting situation that meets the criteria of an SAE should be recorded on the SAE page of the eCRF.

10.4.5. Procedures

All Adverse Events

All AEs (including pregnancy-related AEs throughout pregnancy), regardless of seriousness, severity, or presumed relationship to study vaccination, must be recorded using medical terminology in the source document and the eCRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record in the eCRF their opinion concerning the relationship of the AE to study therapy. All measures required for AE management must be recorded in the source document and reported according to sponsor instructions.

For all studies with an outpatient phase, including open-label studies, the participant must be provided with a "wallet (study) card" and instructed to carry this card with them for the duration of the study indicating the following:

- Study number
- Statement, in the local language(s), that the participant is participating in a clinical study
- Investigator's name and 24-hour contact telephone number
- Local sponsor's name and 24-hour contact telephone number (for medical personnel only)
- Site number
- Participant number

Serious Adverse Events

All SAEs (including pregnancy-related AEs throughout pregnancy), that have not resolved by the end of the study, or that have not resolved upon the participant's discontinuation from the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study vaccine or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (participant or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Suspected transmission of an infectious agent by a medicinal product will be reported as an SAE. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during participation in the study must be reported as an SAE, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or AE (eg, social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study (must be documented in the eCRF). Note: Hospitalizations that were planned before the signing of the ICF, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered SAEs. Any AE that results in a prolongation of the originally planned hospitalization is to be reported as a new SAE.

The cause of death of a participant in a study, whether or not the event is expected or associated with the study vaccine, is considered an SAE.

Information regarding SAEs will be transmitted to the sponsor using an SAE reporting form and safety report form of the eCRF, which must be completed and reviewed by a physician from the study site, and transmitted in a secure manner to the sponsor within 24 hours. The initial and

follow-up reports of an SAE should be transmitted in a secure manner electronically or by facsimile (fax). Telephone reporting should be the exception and the reporter should be asked to complete the appropriate form(s) first.

Adverse Events of Special Interest

AESIs will be carefully monitored during the study by the sponsor. Suspected AESIs must be reported to the sponsor within 24 hours of awareness irrespective of seriousness (ie, serious and non-serious AEs) or causality assessment, following the procedure described above for SAEs and will require enhanced data collection.

10.4.6. Product Quality Complaint Handling

Definition

A PQC is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, ie, any dissatisfaction relative to the identity, quality, durability, reliability, or performance of a distributed product, including its labeling, drug delivery system, or package integrity. A PQC may have an impact on the safety of the product. In addition, it includes any technical complaints, defined as any complaint that indicates a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product or the drug delivery system.

Procedures

All initial PQCs must be reported to the sponsor by the study site personnel within 24 hours after being made aware of the event.

A sample of the suspected product should be maintained under the correct storage conditions until a shipment request is received from the sponsor.

10.4.7. Contacting Sponsor Regarding Safety, Including Product Quality

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding safety issues, PQC, or questions regarding the study are listed in the Contact Information page(s), which will be provided as a separate document.

10.4.8. Neonate Safety Assessment: Apgar Score

Definition: The Apgar score provides an accepted method for reporting the status of the neonate immediately after birth and the response to resuscitation, if needed.

The Apgar score is used to obtain information on the condition of the neonate at birth (including, when available, Apgar scores at 1 minutes and 5 minutes). It is listed in the nonbinding recommendations for pregnancy safety studies, as laid out in the Guidance for Industry by the US Department of Health and Human Services, FDA Center for Drug Evaluation and Research, Draft Guidelines published May 2019 (FDA Guidance 2019).

The Apgar Scoring System: This provides a standardized assessment for infants after delivery. The Apgar score comprises 5 components (1) color; (2) heart rate; (3) reflexes; (4) muscle tone; and (5) respiration. Each of these components is given a score of 0, 1, or 2. The Apgar score is the total of these numbers. The lowest score is 0 and the highest score is 10. A neonate with an Apgar score of 7 or more is considered healthy. The Apgar score can be used to quantify signs of neonatal depression, such as cyanosis or pallor, bradycardia, depressed reflex response to stimulation, hypotonia, and apnea or gasping respiration. The assessment is performed at 1 minute and 5 minutes, in case the infant scores less than 7, other time points would be checked (eg, 10 and 20 minutes) after birth. A copy of the expanded Apgar score reporting form is provided below:

Expanded Apgar score reporting form.

Apgar Score Gestational age_____weeks

Sign	0	1	2	1 minute	5 minute	10 minute	15 minute	20 minute
Color	Blue or Pale	Acrocyanotic	Completely Pink					
Heart rate	Absent	<100 minute	>100 minute					
Reflex irritability	No Response	Grimace	Cry or Active Withdrawal					
Muscle tone	Limp	Some Flexion	Active Motion					
Respiration	Absent	Weak Cry; Hypoventilation	Good, Crying					
			Total					

Comments:	Resuscitation					
	Minutes	1	5	10	15	20
	Oxygen					
	PPV/NCPAP					
	ETT					
	Chest Compressions					
	Epinephrine					

AMERICAN ACADEMY OF PEDIATRICS COMMITTEE ON FETUS AND NEWBORN, and AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS COMMITTEE ON OBSTETRIC PRACTICE Pediatrics 2015;136:819-822

10.5. Appendix 5: Contraceptive Guidance

At the time of consenting, participants should agree to practicing an acceptable effective method of contraception postpartum and agree to remain on such a method of contraception following the birth of the infant until 3 months after administration of the study vaccine.:

Contraceptive (birth control) use by a participant of childbearing potential should be consistent with local regulations regarding the acceptable methods of contraception for those participating in clinical studies.

Participant who will receive a booster of study vaccine postpartum, should be using a highly effective method of contraception and agree to remain on such a method of contraception following the birth of the infant until 3 months after the booster of study vaccine. Use of hormonal contraception should start at least 28 days before the administration of study vaccine. Highly effective methods for this study include:

1. hormonal contraception*
    a. combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, or transdermal)
    b. progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, or implantable)
2. intrauterine device (IUD)
3. intrauterine hormone-releasing system (IUS)
4. bilateral tubal occlusion/ligation procedure
5. vasectomized partner (the vasectomized partner should be the sole partner for that participant)
6. sexual abstinence**

** or per physician preference/local standard of care in a woman who is breastfeeding*

***Sexual abstinence is considered an effective method **only** if defined as refraining from heterosexual intercourse from signing the informed consent until 3 months after the last dose of study vaccine. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.*

10.6. Appendix 6: Toxicity Grading Scales

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

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

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) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
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.

10.7. Appendix 7: Multisystem Inflammatory Syndrome in Children

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. Patients 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[a], laboratory evidence of inflammation[b], 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.

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

[b] 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.

10.8. Appendix 8: Case Definitions for COVID-19

10.8.1. Adults Case Definition for Moderate to Severe/Critical COVID-19

Case Definition for Moderate COVID-19

- A SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample (eg, nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample.

AND at any time during the course of observation[a]:

Any 1 of the following new or worsening signs or symptoms:

- Respiratory rate ≥20 breaths/minute
- Abnormal saturation of oxygen (SpO_2) but still >93% on room air at sea level*
- Clinical or radiologic evidence of pneumonia
- Radiologic evidence of DVT
- Shortness of breath or difficulty breathing

OR

Any 2 of the following new or worsening signs or symptoms:

- Fever (≥38.0°C or ≥100.4°F)
- Heart rate ≥90 beats/minute
- Shaking chills or rigors
- Sore throat
- Cough
- Malaise as evidenced by 1 or more of the following**:
  - Loss of appetite
  - Generally unwell
  - Fatigue
  - Physical weakness
- Headache
- Muscle pain (myalgia)
- Gastrointestinal symptoms (diarrhea, vomiting, nausea, abdominal pain)
- New or changing olfactory or taste disorders
- Red or bruised looking feet or toes

SpO_2 criteria will be adjusted according to altitude.
** Having 2 or more elements of a symptom (eg, vomiting and diarrhea or fatigue and loss of appetite) is counted only as 1 symptom for the case definition. To meet the case definition, a participant would need to have at least 2 different symptoms.

[a] Participants will be asked to undertake the COVID-19 procedures until 14 days after symptom onset (COVID-19 Day 15) or until **resolution of the COVID-19 episode**, whichever comes last (see Section 8.1.2).

Case Definition for Severe/Critical COVID-19

- A SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample (eg, nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample

AND any 1 of the following at any time during the course of observation[a]:

- Clinical signs at rest indicative of severe systemic illness (respiratory rate ≥30 breaths/minute, heart rate ≥125 beats/minute, oxygen saturation [SpO_2] ≤93% on room air at sea level*, or partial pressure of oxygen/fraction of inspired oxygen [PaO_2/FiO_2] <300 mm Hg)

  * SpO_2 criteria will be adjusted according to altitude.
- Respiratory failure (defined as needing high-flow oxygen, non-invasive ventilation, mechanical ventilation, or extracorporeal membrane oxygenation [ECMO])
- Evidence of shock (defined as systolic blood pressure <90 mm Hg, diastolic blood pressure <60 mm Hg, or requiring vasopressors)
- Significant acute renal, hepatic, or neurologic dysfunction
- Admission to the ICU
- Death

10.8.2. Adult Case Definition for Mild COVID-19

- A SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample (eg, nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample;

AND at any time during the course of observation[a]:

- One of the following symptoms: fever (≥38.0°C or ≥100.4°F), sore throat, malaise (loss of appetite, generally unwell, fatigue, physical weakness), headache, muscle pain (myalgia), gastrointestinal symptoms, cough, chest congestion, runny nose, wheezing, skin rash, eye irritation or discharge, chills, new or changing olfactory or taste disorders, red or bruised looking feet or toes, or shaking chills or rigors.

A case is considered clinically mild when it meets the above case definition but not the moderate to severe/critical definition in Section 10.8.1.

[a] Participants will be asked to undertake the COVID-19 procedures until 14 days after symptom onset (COVID-19 Day 15) or until **resolution of the COVID-19 episode**, whichever comes last (see Section 8.1.2).

10.8.3. Adult US FDA Harmonized Case Definition for COVID-19

If a participant presents with symptoms as those listed by the US FDA harmonized case definition (CDC 2020c), the investigator (or designated medically trained clinician) should assess if these are suggestive of COVID-19:

- A SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample (eg, nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample; AND
- COVID-19 symptoms consistent with those defined by the US FDA harmonized case definition (CDC 2020c) at the time of finalization of this protocol: fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea.

10.8.4. Neonates and Infants Case Definition for Moderate to Severe/Critical COVID-19

Case Definition for Moderate COVID-19

- A SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample (eg, nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample.

AND at any time during the course of observation[a]:

Any 1 of the following new or worsening signs or symptoms:

- Abnormal respiratory rate[b]
  - but still ≤70 breaths/minute for neonates and infants <6 months of age
  - but still ≤60 breaths/minute for infants ≥6 months of age
- Abnormal saturation of oxygen (SpO_2) but still >90% on room air at sea level*
- Clinical or radiologic evidence of pneumonia
- Intercostal retractions (in-drawing)
- Heart rate ≥160 beats/minute for neonates (≤28 days of age) and ≥140 beats/minute for infants >28 days to ≤12 months of age)
- Rash
- Bloodshot eyes

OR

Any 2 of the following new or worsening signs or symptoms:

- Fever (≥38.0°C or ≥100.4°F)
- Shaking chills or rigors
- Wheezing
- Cough
- Malaise as evidenced by 1 or more of the following**:
  - Poor feeding
  - Moodiness (frequent crying, irritability)
  - Lethargy/tiredness
- Gastrointestinal symptoms** (diarrhea, vomiting)
- Red or bruised looking feet or toes

* SpO_2 criteria will be adjusted according to altitude.
** Having 2 or more elements of a symptom (eg, vomiting and diarrhea or lethargy and loss of appetite/poor feeding) is counted only as 1 symptom for the case definition. To meet the case definition, a participant would need to have at least 2 different symptoms.

[a] Participants will be asked to undertake the COVID-19 procedures until 14 days after symptom onset (COVID-19 Day 15) or until **resolution of the COVID-19 episode**, whichever comes last (see Section 8.1.2).

[b] Per Golan-Tripto I et al 2018.

Case Definition for Severe/Critical COVID-19

- A SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample (eg, nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample

AND any 1 of the following at any time during the course of observation[a]:

- Clinical signs at rest indicative of severe systemic illness (respiratory rate ≥71 breaths/minute for neonates and infants <6 months of age and ≥61 breaths/minute for infants ≥6 months of age[b], heart rate ≥180 beats/minute[c], SpO_2 ≤89% on room air at sea level*, or PaO_2/FiO_2 <300 mm Hg)

  *SpO_2 criteria will be adjusted according to altitude
- Respiratory failure (defined as needing high-flow oxygen, non-invasive ventilation, mechanical ventilation, or ECMO)
- Evidence of shock (defined as systolic blood pressure <65mmHg to 75mmHg for neonates and <100 mm Hg for infants, or requiring vasopressors)
- Significant acute renal, hepatic, or neurologic dysfunction
- Multisystem inflammatory syndrome (MIS-C)[d]

  symptoms: fever, vomiting, diarrhea, rash, bloodshot eyes, feeling extra tired

  trouble breathing, inability to stay awake, bluish lips or face
- Admission to the Pediatric ICU
- Death

[a] Participants will be asked to undertake the COVID-19 procedures until 14 days after symptom onset (COVID-19 Day 15) or until **resolution of the COVID-19 episode**, whichever comes last (see Section 8.1.2).

[b] Per Golan-Tripto I et al 2018.

[c] Per Goldstein B et al. 2005

[d] Multisystem Inflammatory Syndrome in Children (MIS-C) associated with COVID-19 per CDC website: https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/children/mis-c.html. Accessed on 12 Nov 2020. Note: the list of symptoms was adapted for neonates and infants.

10.8.5. Neonates Case Definition for Mild COVID-19

- A SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample (eg, nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample;

AND at any time during the course of observation[a]:

- One of the following symptoms: fever (≥38.0°C or ≥100.4°F), poor feeding, tachycardia, lethargy, diarrhea, vomiting, cough, or rhinorrhea.

A case is considered clinically mild when it meets the above case definition but not the moderate to severe/critical definition in Section 10.8.4.

10.8.6. Infants Case Definition for Mild COVID-19

- A SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample (eg, nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample;

AND at any time during the course of observation[a]:

- [b]One of the following symptoms: fever (≥38.0°C or ≥100.4°F) or chills, poor appetite or poor feeding, mood behaviors (frequent crying, irritability), lethargy/tiredness, diarrhea, vomiting, cough, wheezing, tachypnea, nasal congestion or runny nose,

A case is considered clinically mild when it meets the above case definition but not the moderate to severe/critical definition in Section 10.8.4.

[a] Participants will be asked to undertake the COVID-19 procedures until 14 days after symptom onset (COVID-19 Day 15) or until **resolution of the COVID-19 episode**, whichever comes last (see Section 8.1.2).

[b] Source: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html?CDC AA refVal=https%3A%2F%2Fwww.cdc.gov%2 Fcoronavirus% 2F2019-ncov%2Fneed-extra-precautions%2Fgroups-at-higher-risk.html. Accessed 03 December 2020.

10.9. Appendix 9: Summary of Guidance from CDC Website on Underlying Medical Conditions That Lead or Might Lead to Increased Risk for Severe Illness From COVID-19

10.9.1. Appendix 9A: CDC Website[a] Underlying Medical Conditions for Adults

People of any age with the conditions listed below are more likely to get severely ill from COVID-19. COVID-19 vaccines (initial dose and boosters) and preventive measures for COVID-19 are essential.

Approved and authorized COVID-19 vaccines (initial dose and boosters) are safe and effective and should be administered to people at higher risk including people with underlying medical conditions.

This list does not include all possible conditions that place a higher risk of severe illness from COVID-19. Severe illness from COVID-19 is defined as hospitalization, admission to the ICU, intubation or mechanical ventilation, or death.

Adults of any age with the following conditions are at increased risk of severe illness from COVID-19:

- Cancer
- Chronic kidney disease
- Chronic liver disease
- COPD (chronic obstructive pulmonary disease)
- Heart conditions, such as heart failure, coronary artery disease or cardiomyopathies
- Immunocompromised state (weakened immune system) from solid organ transplant
- Obesity (BMI of 30 kg/m^2 or higher but < 40 kg/m^2)
- Severe Obesity (BMI ≥ 40 kg/m^2)
- Pregnancy
- Stroke or cerebrovascular disease
- Sickle cell disease
- Smoking
- Diabetes (Type 1 or Type 2)

COVID-19 is a new disease. Data and information are still currently under investigation to better understand the impact of underlying medical conditions and actual risks for severe illness from COVID-19:

- Asthma (moderate to severe)
- Cerebrovascular disease (affects blood vessels and blood supply to the brain)

- Cystic fibrosis
- Hypertension or high blood pressure
- Immunocompromised state (weakened immune system) from blood or bone marrow transplant, immune deficiencies, HIV, use of corticosteroids, or use of other immune weakening medicines
- Neurologic conditions, such as dementia
- Liver disease
- Overweight (BMI > 25 kg/m^2, but < 30 kg/m^2)
- Pulmonary fibrosis (having damaged or scarred lung tissues)
- Thalassemia (a type of blood disorder)
- Type 1 diabetes mellitus

10.9.2. Appendix 9B: CDC Website Underlying Medical Conditions for Children and Teens

CDC and partners are investigating a rare but serious medical condition associated with COVID-19 in children called Multisystem Inflammatory Syndrome in Children (MIS-C). We do not yet know what causes MIS-C and who is at increased risk for developing it.

Babies under 1 year old might be more likely to have severe illness from COVID-19. Other children, regardless of age, with the following underlying medical conditions might also be at increased risk of severe illness compared to other children:

- Asthma or chronic lung disease
- Diabetes
- Genetic, neurologic, or metabolic conditions
- Heart disease since birth
- Immunosuppression (weakened immune system due to certain medical conditions or being on medications that weaken the immune system)
- Medical complexity (children with multiple chronic conditions that affect many parts of the body who are often dependent on technology and other significant supports for daily life)
- Obesity

This list does not include every underlying condition that might increase the risk for severe illness in children. As more information becomes available, CDC will continue to update and share information about risk for severe illness among children.

10.10. Appendix 10: Symptoms of Coronavirus (US Centers for Disease Control and Prevention)

10.10.1. Appendix 10A: Symptoms of Coronavirus (US Centers for Disease Control and Prevention) in Adults

The following extract shows symptoms of coronavirus infection in adults as listed on the US CDC website dated 09 November 2020 and were still accurate at the time of finalization of this protocol:

(https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html).

People with COVID-19 have had a wide range of symptoms reported - ranging from mild symptoms to severe illness. Symptoms may appear **2-14 days after exposure to the virus**. People with these symptoms may have COVID-19:

- Fever or chills
- Cough
- Shortness of breath or difficulty breathing
- Fatigue
- Muscle or body aches
- Headache
- New loss of taste or smell
- Sore throat
- Congestion or runny nose
- Nausea or vomiting
- Diarrhea

10.10.2. Appendix 10B: Symptoms of Coronavirus (US Centers for Disease Control and Prevention) in Neonates

The following extract shows symptoms of coronavirus infection in neonates as listed on the US CDC website dated 23 October 2020 and were still accurate at the time of finalization of this protocol:

(https://www.cdc.gov/coronavirus/2019-ncov/hcp/caring-for-newborns.html)

Note: Current evidence suggests that SARS-CoV-2 infections in neonates are uncommon. If neonates do become infected, the majority have either asymptomatic infections or mild disease (ie, do not require respiratory support), and they recover. Severe illness in neonates, including illness requiring mechanical ventilation, has been reported but appears to be rare. Neonates with underlying medical conditions and preterm infants (<37 weeks gestational age) may be at higher risk of severe illness from COVID-19.

Reported signs among neonates with SARS-CoV-2 infection include:

- Fever
- Lethargy
- Rhinorrhea
- Cough
- Tachypnea
- Increased work of breathing
- Vomiting
- Diarrhea
- Poor feeding

The extent to which SARS-CoV-2 infection contributed to the reported signs of infection and complications is unclear, as many of these findings are common in term and preterm infants for other reasons (eg, transient tachypnea of the neonate, neonatal respiratory distress syndrome).

10.10.3. Appendix 10C: Symptoms of Coronavirus (US Centers for Disease Control and Prevention) in Children

The following extract shows symptoms of coronavirus infection in infants and children as listed on the US CDC website dated 17 September 2020 (CDC 2020g) and were still accurate at the time of finalization of this protocol:

(https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/children/symptoms.html)

The most common symptoms of COVID-19 in children are fever and cough.

The symptoms of COVID-19 are similar in adults and children and can look like other common illnesses, like colds, strep throat, or allergies. The most common symptoms of COVID-19 in children are fever and cough, but children may have any of these signs or symptoms of COVID-19:

- Fever or chills
- Cough
- Nasal congestion or runny nose
- New loss of taste or smell
- Sore throat
- Shortness of breath or difficulty breathing
- Diarrhea
- Nausea or vomiting
- Stomachache
- Tiredness
- Headache
- Muscle or body aches
- Poor appetite or poor feeding, especially in babies under 1 year old.

10.11. Appendix 11: Thrombosis with Thrombocytopenia (TTS) AESI Form

The form below represents the type of information that may be collected in case of a suspected AESI in order to help adjudicate whether the event is a case of post-vaccination TTS. Additional data may be requested by the sponsor for investigation of the event.

Adverse Event of Special Interest Questionnaire (AESIQ) for Thromboembolism with Thrombocytopenia Syndrome

Date of Report: [dd-MMM-yyyy]

1. Adverse Event Description

Participant's clinical signs and symptoms

☐ Leg/Calf Oedema	☐ Pain in Leg/Calf	☐ Haemoptysis
☐ Dyspnea	☐ Chest Pain/Discomfort	☐ Syncope
☐ Tachypnoea	☐ Tachycardia	☐ Cough
☐ Loss of consciousness	☐ Headache	☐ Seizure
☐ Visual impairment	☐ Weakness	☐ Impaired speech
☐ Confusional state	☐ Paresthesia	☐ Gait disturbance

☐ Other symptoms:

Was patient on VTE prophylaxis? ☐ No ☐ Yes, details:

2. Medical History and Concurrent Conditions

Provide details:

Is the participant overweight or have obesity?	☐ No	☐ Yes
If available, please provide: Height Weight BMI		
Does the participant have a sedentary lifestyle[cc]?	☐ No	☐ Yes – details:
Has the participant been in a sitting position for long periods of time prior to the event?	☐ No	☐ Yes – details:
Is there a current history of smoking (active or passive)?	☐ No	☐ Yes – details:
Is there a prior history of smoking (active or passive)?	☐ No	☐ Yes – details:
Does the participant have a prior history of:		
Cancer	☐ No	☐ Yes – details:
Autoimmune disease (i.e., collagen-vascular disease, inflammatory bowel disease) or myeloproliferative disease?	☐ No	☐ Yes – details:
Clotting disorder or a hypercoagulable state	☐ No	☐ Yes – details:
Varicose veins	☐ No	☐ Yes – details:
Trauma to the involved leg or pelvis	☐ No	☐ Yes – details:
DVT/PE or other VTE	☐ No	☐ Yes – details:
Blood transfusion	☐ No	☐ Yes – details:
Cardiovascular disease	☐ No	☐ Yes – details:
If the participant has experienced a previous thrombotic event, address the following: 1. Date (or estimate) 2. Provide brief description of the nature of the event 3. Provide brief description of the treatment of the event 4. Note any residual manifestations of the event.		
If the patient has experienced more than one previous thrombotic event, please list other events.		
Was the (female) participant pregnant at the time of event?	☐ No	☐ Yes – details:

Does the participant has any of genetic risk factors:

☐ Dysfibrinogenemia ☐ Antiphospholipid syndrome ☐ Factor V Leiden mutation
☐ Protein C or S deficiency ☐ Elevated factor VIII levels ☐ Anti-thrombin deficiency
☐ Hyperhomocysteinemia ☐ Prothrombin gene mutation ☐ Blood-clotting disorder
☐ Thrombophilia

Does the participant have any acquired risk factors:

☐ Reduced mobility (paralysis, paresis, travel etc.) ☐ Recent surgery
☐ Indwelling central venous catheters ☐ Recent trauma
☐ Recent discontinuation of anticoagulants (e.g., heparin, warfarin, DOACs)
☐ Hormone replacement therapy (including contraceptives)
☐ Phlebitis ☐ Lupus
☐ Inflammatory bowel disease ☐ Myeloproliferative disorders
☐ Diabetes mellitus ☐ Hyperlipidemia

☐ Hypertension ☐ Dehydration

☐ Other significant medical comorbidities or risk factors for DVT, specify:

If yes to any of the above, provide details:

Provide Well's score, if calculated:

3. Relevant Results of Diagnostic Tests Including Laboratory Tests, Imaging, Biopsies, etc. (Note the Levels/Conclusion, Date Performed, Normal Ranges As Well As Any Other Details. Alternatively, Attach Full Reports of the Diagnostic Tests.)

Diagnostic Test	Results at baseline or prior to use of product (Include date and value/details)	Test results after use of product (Include date and value/details)
CBC with smear (microscopic evaluation)		
ESR		
Platelet count		
Antibodies to platelet factor 4 (PF4)		
Fibrinogen levels		
Clauss fibrinogen assay		
D-Dimer levels		
Clotting Profile (PT, aPTT- prior to an anti-coagulation treatment)		
Thrombin time (Bovine) Plasma		
Prothrombin		
Antithrombin activity		
Factor V Leiden		
Protein C activity		
Protein S activity		
C-reactive protein		
Homocystein levels		
Dilute Russells Viper Venom Time (DRVVT), Plasma		
Activated Protein C Resistance V (APCRV), Plasma		
Thrombophilia interpretation		
Anti-cardiolipin antibodies (IgG and IgM) or beta-2 glycoproteins antibodies		
Antiphospholipid antibodies (IgG and IgM)		
Lupus anticoagulant		
Heparin antibodies		
ANA and ANCA		
IL6 levels		
ADAMTS13 Activity Assay		

Diagnostic Test	Results at baseline or prior to use of product (Include date and value/details)	Test results after use of product (Include date and value/details)
Ceruloplasmin		
Direct Coombs test		
Complement C3, C4		
MethylenetetraHydrofolate reductase gene mutation		
Prothrombin gene mutation (G20210A)		
Occult blood in stool		
COVID-19 test		
Troponins		
Brain Natriuretic Peptide		
Arterial Blood Gases		
Chest X-Ray		
Electrocardiography		
Echocardiography		
Duplex Ultrasonography		
MRI scan		
CT scan		
Contrast Venography		
Pulmonary Angiography		
Ventilation-Perfusion Scanning		

Provide details of any additional diagnostic results:

10.12. Appendix 12: Thrombotic Events to be Reported as Suspected AESIs

At the time of writing the current protocol Amendment 3, the list of thrombotic events to be reported to the sponsor as suspected AESIs is provided below. Further guidance may become available on thrombotic events of interest.[a]

- MedDRA PTs for large vessel thrombosis and embolism:
    - Aortic embolus, aortic thrombosis, aseptic cavernous sinus thrombosis, brain stem embolism, brain stem thrombosis, carotid arterial embolus, carotid artery thrombosis, cavernous sinus thrombosis, cerebral artery thrombosis, CVST, cerebral venous thrombosis, superior sagittal sinus thrombosis, transverse sinus thrombosis, mesenteric artery embolism, mesenteric artery thrombosis, mesenteric vein thrombosis, splenic artery thrombosis, splenic embolism, splenic thrombosis, thrombosis mesenteric vessel, visceral venous thrombosis, hepatic artery embolism, hepatic artery thrombosis, hepatic vein embolism, hepatic vein thrombosis, portal vein embolism, portal vein thrombosis, portosplenomesenteric venous thrombosis, splenic vein thrombosis, spontaneous HIT syndrome, femoral artery embolism, iliac artery embolism, jugular vein embolism, jugular vein thrombosis, subclavian artery embolism, subclavian vein thrombosis, obstetrical pulmonary embolism, pulmonary artery thrombosis, pulmonary thrombosis, pulmonary venous thrombosis, renal artery thrombosis, renal embolism, renal vein embolism, renal vein thrombosis, brachiocephalic vein thrombosis, vena cava embolism, vena cava thrombosis, truncus coeliacus thrombosis.
- MedDRA PTs for more common thrombotic events:
    - Axillary vein thrombosis, DVT, pulmonary embolism, MedDRA PTs for acute myocardial infarction,[b] MedDRA PTs for stroke[b]

[a] Source: Shimabukuro T. CDC COVID-19 Vaccine Task Force. Thrombosis with thrombocytopenia syndrome (TTS) following Janssen COVID-19 vaccine. Advisory Committee on Immunization Practices (ACIP). April 23, 2021. https://www.cdc.gov/vaccines/acip/meetings/slides-2021-04-23.html.

[b] *Vaccine Adverse Event Reporting System (VAERS) Standard Operating Procedures for COVID-19 (as of 29 January 2021) https://www.cdc.gov/vaccinesafety/pdf/VAERS-v2-SOP.pdf

10.13. Appendix 13: Visit Guidance in Case of Pregnancy Termination Before the Completion of the Applicable Study Period Visits

General Guidance:

- In the situation of a completion/termination of the pregnancy before the completion of the applicable Study Period visits, the participant should follow the visits scheduled in the 'Study Period' (except for the Monthly phone calls) AND the 'Follow-up' section of the schedule of activities.
- If the pregnancy is completed/ terminated before the completion of the Day 29 it should be judged based on the participants health and safety if these visits can be performed. These visits are associated to our primary objective and therefore of high interest to be completed.
- If the delivery takes place in a hospital where the site staff does not have privileges, it is expected that medical records are collected to collect the applicable protocol required information.

Randomization	General timing of birth	Detailed timing of birth	Guidance for completion of visits	Protocol language
1-dose schedule	Birth before completion Study Period visits Vaccination 1	Before start visit window of Day 29	Day 8	If not yet performed, visit can be skipped. Please perform diary review with participant as soon as feasible (phone is acceptable)
			Day 15	
			Day 29	This visit should be performed, based on assessment of participant health
			Monthly phone calls	Not applicable
		During visit window of Day 29	Day 29	If not yet performed, visit can be skipped, provided that PP1 has occurred. If Day 29 nor PP1 visit were performed, Day 29 is recommended to be performed as soon as possible based on assessment of participant health, as long as it does not overlap with the window of the next scheduled visit.
			Monthly phone calls	Not applicable
		After Day 29	Monthly phone calls	Not applicable

10.14. Appendix 14: Protocol Amendment History

Amendment 6 (05 May 2022)

Overall Rationale for the Amendment:

Safety data with Ad26.COV2.S when administered within 3 months before pregnancy as well as during pregnancy have shown no safety concerns in the mother or child in over 500 reported pregnancies, with over 100 reported pregnancy outcomes. In addition safety data with other Janssen adenovirus type 26 (Ad26)-based vaccines when administered within 3 months before pregnancy as well as during pregnancy have shown no evidence of an increased risk of adverse outcomes in the mother or child in over 2200 reported pregnancies, with over 1400 reported pregnancy outcomes (IB 2022). Due to the safety data accrued to date on the use of Ad26.COV2.S specifically and Ad26-based vaccines in general and taking into account the rapid progression of national vaccination campaigns, which makes it difficult to recruit 'non-previously vaccinated' (vaccine naïve) pregnant women, the following changes were made to facilitate enrollment: the possibility to recruit previously vaccinated and boosted women with several vaccination regimens, and in order to complete the study to provide reactogenicity and immunogenicity data during pregnancy the sample size has been decreased from 400 to approximately 240 participants.

In the current fluid coronavirus disease-2019 (COVID-19) environment, emergent recommendations for primary vaccination schemes and booster vaccination campaigns have reduced the pool of eligible participants for the study. Therefore, study groups have been modified to allow enrollment of participants previously vaccinated and those who had received booster doses up to 4 months prior to the study vaccination.

Changes made to the clinical protocol of study VAC31518COV2004 as part of Amendment 6 are listed below, including the rationale for each change and a list of all applicable sections. Changes made as part of Protocol Amendments 1 to 5 are listed in Appendix 10.14.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.2 Schema 4.1 Overall Design 6.3 Preparation/Handling/Storage/ Accountability 9.2 Sample Size Determination 9.2.1 Immunogenicity 9.5.2 Interim Analysis 2	The study design has been amended so that the overall target number of participants is reduced from N=400 to N=240 participants, preferably distributed equally across groups but at least 40 participants in Groups 1-3 and at least 25 participants in Group 4. The number of remaining participants (excluding Sentinel and Safety Cohorts) is N=218.	See overall rationale for amendment.
1.1 Synopsis 1.2 Schema 1.3 Schedule of Activities 4.1 Overall Design 5.1 Inclusion Criteria 5.2 Exclusion Criteria 6.8 Prestudy and Concomitant Therapy	Inclusion criterion #11 and Exclusion criterion #13 have been updated to reduce the timeframe of last vaccination prior to study vaccination to at least 4 months.	To align with recent recommendations for booster doses in certain countries.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 4.1 Overall Design 5.1 Inclusion Criteria	Inclusion criterion #11 updated to clarify the inclusion of participants that were vaccinated with a COVID-19 vaccine beyond primary regimen for Groups 1-3. Group descriptions have been updated and now indicates Groups 1 and 2 are including participants that were also boosted with the homologous vaccine. Group 3 would also be recruiting boosted participants, irrespective of the vaccine and regimen.	Change in eligibility criteria to allow inclusion of participants that were vaccinated with a COVID-19 vaccine beyond primary regimen as most potential study participants have now received at least one booster vaccination.
1.3 Schedule of Activities	SARS-CoV-2 vaccination history in the Schedule of Activities tables and footnotes has been updated to COVID-19 vaccination history to align with text in the body.	To align with text in the body of the protocol.
1.1 Synopsis 1.3.1.2 Vaccine Naïve Adult Participants (Group 4, Including Sentinel and, Safety Cohorts): Booster Vaccination Schedule 2.3.3 Benefit-Risk Assessment for Study Participation 4.1 Overall Design 8.2.5 Breast Milk Production Outcomes 8.3.2 Method of Detecting Adverse Events, Adverse Events of Special Interest, Serious Adverse Events and Medically-attended Adverse Events	Potential changes in (postpartum) breast milk production (reduction) has been added as part of the safety evaluation for Group 4 participants that receive a booster. Changes in breast milk production has been added to solicited systemic adverse events.	Potential subjective changes in breast milk production in lactating participants, receiving the Janssen vaccine as a booster postpartum, is added as part of the safety evaluations.
1.1 Synopsis 1.3 Schedule of Activities 3 OBJECTIVES AND ENDPOINTS 4.1 Overall Design 8 STUDY ASSESSMENTS AND PROCEDURES 8.1 Immunogenicity Assessments 9.2 Sample Size Determination 9.4.3 Exploratory Endpoints(s)	Wording updated regarding the PBMC subset being specific to any group and not vaccine naïve only. The PBMC subset has been updated to approximately 25 adult participants per group, if operationally feasible. Additionally, it was clarified that the objective relating to PBMC sampling will only be tested if PBMC collection is feasible in a sufficient proportion of participants. Table 4 has been updated when the probabilities of observing at least one Th2 event given true Th2 rates if N=25.	PBMC collection has been adjusted to include all groups in the study (if feasible), to additionally allow for an assessment of cellular immune responses in participants who are previously vaccinated, as these are a relevant population in the current world setting.
2.3.1 Risks Related to Study Participation 8.5 Genetics and Pharmacogenomics	Genetic testing replaced with genomic research to use language that is inclusive of the intended gene expression analysis by RNAseq transcript profiling.	Correction.
5.1 Inclusion Criteria	Inclusion Criterion #3 Number of CD4 cells required is updated to ≥300 cells/µL.	Alignment across Ad26.COV2.S protocols

Section Number and Name	Description of Change	Brief Rationale
5.1 Inclusion Criteria	Inclusion Criterion #3, clarifying that if a potential participant does not have HIV viral load and CD4 cell count data in their medical records from the last 6 months efforts will be made to obtain the necessary data for potential entry into the study. A laboratory result within 6 months of screening does not need to be repeated.	Clarification
5.2 Exclusion Criteria 6.8 Prestudy and Concomitant Therapy	Wording for Exclusion Criterion #8 was modified to indicate the definition of immunosuppressive dose as stated per (>20 mg prednisone or equivalent daily for 2 consecutive weeks). Intraarticular steroids have been removed from this sentence.	Alignment across Ad26.COV2.S protocols. Intraarticular steroids are typically use as a single shot indicated for pain and inflammation reliever. The criteria defines an immunosuppressive steroid dose is considered to be >20 mg prednisone or equivalent daily for 2 consecutive weeks. That definition is not consistent with the typical frequency of use in clinical practice.
1.1 Synopsis 2 INTRODUCTION	Updated text to define the clinical isolate of Wuhan, 2019.	Clarification
1.1 Synopsis 3 OBJECTIVES AND ENDPOINTS 9.2.2 Safety 9.4.2 Primary/Secondary Endpoint(s)	Endpoint has been revised to include “or without” complications to term neonate and preterm neonate.	Correction
1.3 Schedule of Activities 8.2.1 Physical Examinations	Post-pregnancy physical examination has been removed and aligned with the targeted physical examinations.	Correction
1.1 Synopsis 1.2 Schema 1.3 Schedule of Activities 4.1 Overall Design 6.2 Booster Vaccination	It has been clarified for booster vaccinations the vaccine naïve participants (Group 4) must not be pregnant or have received another COVID-19 vaccine (eg, national immunization program) If so, the participant is not allowed to receive the booster dose.	Correction
6.9 Study Vaccination Pausing Rules	Clarified the medical monitor does not coordinate meetings with IDMC but will trigger the process for pausing.	Clarification
1.1 Synopsis 8.1.1 Immunogenicity Assessments	Clarified blood sampling information.	Clarification

Section Number and Name	Description of Change	Brief Rationale
8.1.2 Procedures in the Event of (Suspected) COVID-19	Clarified if a clinic visit is not feasible a home may be allowed if allowed per local regulations and transfer of a nasal swab to the study site as soon as possible after collection (within 3 days).	Clarification
9.2 Sample Size Determination	The probability of observing at least one adverse event given a true adverse event incidence table (Table 3) has been updated to reflect a smaller population.	Clarification
Throughout the protocol	Minor errors and inconsistencies were corrected, and minor clarifications were added throughout the protocol.	Correction of minor errors and inconsistencies. Addition of minor clarifications.

Amendment 5 (18 March 2022)

Overall Rationale for the Amendment:

Modify the total sample size for the safety and reactogenicity evaluation of the Sentinels (n=5) and larger Safety Cohort (n=17) from N=25 to N=22. Recruitment of vaccine naïve pregnant participants in the Safety Cohort turned out to be severely impacted by ongoing vaccination campaigns. The sponsor approached the IDMC to discuss whether the safety data accrued on 22 participants from the Sentinel and Safety Cohorts (up to 26 February 2022) would allow a thorough and educated assessment by the IDMC. Due to a longer recruitment time, in addition the originally planned at Day 8 (7 days post-vaccination) data, meaningful longer term safety data for the 22 participants had been collected to support the safety assessment).

Based on the review of the Sentinel and Safety Cohorts data (N=22), the IDMC advised that the safety and reactogenicity profile of Ad26.COV2.S at 5×10^{10} vp was considered acceptable and no safety concerns were identified in adult pregnant women. The remaining 375 planned participants will receive 1 dose of Ad26.COV2.S at the 5×10^{10} vp at study entry. Subsequently, the 2 dose regimen of Ad26.COV2.S at the lower dose level of 2.5×10^{10} vp was be removed from the main study as no longer needed.

Changes made to the clinical protocol of study VAC31518COV2004 as part of Amendment 5 are listed below, including the rationale for each change and a list of all applicable sections. Changes made as part of Protocol Amendments 1 to 4 are listed in Appendix 10.14.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.2 Schema 2 INTRODUCTION 4.1 Overall Design 6.3 Preparation/Handling/Storage/ Accountability 6.5 Dose Modification	Text updated for the combined number of vaccine naïve adult pregnant women in the Sentinel and Safety Cohorts from N=25 to N=22. Text updated for the number of participants in the Safety Cohort only from n=20 to n=17. All applicable sections have been updated accordingly.	Recruitment challenges of vaccine naïve pregnant participants

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.2 Schema 1.3 Schedule of Activities 2 INTRODUCTION 3 OBJECTIVES AND ENDPOINTS 4.1 Overall Design (including Figure 2) 6.1 Study Vaccinations Administered 6.2 Booster Vaccination 6.3 Preparation/Handling/Storage/ Accountability 6.5 Dose Modification 6.8 Prestudy and Concomitant Therapy 7.1 Discontinuation of Study Vaccination 7.2 Delay or Discontinuation of Vaccination due to Pregnancy-Specific Complications 8 STUDY ASSESSMENTS AND PROCEDURES 8.2.1 Physical Examinations 9.2.1 Immunogenicity 9.2.2 Safety 9.4.2 Primary/Secondary Endpoints 9.4.3 Exploratory Endpoints 9.5.2 Interim Analysis 2 10.13 Appendix 13 10.5 Contraceptive Guidance	Text updated to indicate that following IDMC review of the Sentinel and Safety Cohorts (N=22), the safety and reactogenicity profile of 1 dose of Ad26.COV2.S at 5×10^{10} vp was considered acceptable and no safety concerns were identified. The remaining 375 planned participants will receive 1 dose of Ad26.COV2.S at 5×10^{10} vp. Removed information regarding 2 doses of Ad26.COV2.S at 2.5×10^{10} vp, which included deleting Section 1.3.3. Subsequent subsections were renumbered accordingly. Removed information regarding second dose or second vaccination. All applicable sections have been updated accordingly.	See overall rationale for amendment
1.3 Schedule of Activities	It has been clarified for booster vaccinations the vaccine naïve participants (Group 4) must not have received another COVID-19 vaccine (eg, national immunization program) If so, the participant is not allowed to receive the booster dose.	Clarification
1.1 Synopsis 3 OBJECTIVES AND ENDPOINTS 8.3.1 Time Period and Frequency for Collecting Information Relating to Adverse Events, Adverse Events of Special Interest, Serious Adverse Events and Medically-attended Adverse Events 8.3.3 Follow-up of Adverse Events, Serious Adverse Events, Adverse Events of Special Interest, Pregnancy-related AEs and Medically-attended Adverse Events 9.2.2 Safety 9.4.2 Primary/Secondary Endpoints	To align with study objectives, pregnancy-related AEs throughout pregnancy have been added. All applicable sections have been updated accordingly.	Clarification
1.1 Synopsis 1.3 Schedule of Activities 4.1 Overall Design 8.1.1 Immunogenicity Assessments	It has been clarified biomarker analysis will be in PAXgene® tubes.	Clarification
1.1 Synopsis 8.3.1 Time Period and Frequency for Collecting Information Relating to Adverse Events, Adverse Events of Special Interest, Serious Adverse Events and Medically-attended Adverse Events	It has been clarified the investigator's assessment of ongoing AEs at the time of each participant's last visit should be documented and closed in the participant's medical chart.	Clarification

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.3 Schedule of Activities	It has been clarified only the overall Apgar score is required to be documented in the eDC.	Clarification
1.1 Synopsis 9.2.1 Immunogenicity 9.4.1 General Considerations 9.4.2 Primary/Secondary Endpoints	It has been clarified that baseline serostatus refers to SARS-CoV-2 baseline serostatus.	Clarification
1.3 Schedule of Activities	It has been clarified in schedule of activities the physical examination is a full physical examination. It has been clarified that the physical examination at PP1 will be a targeted physical examination.	Clarification
1.1 Synopsis 1.3.4. Procedures for Adult Participants With (Suspected) COVID-19, 1.3.5. Procedures for Neonates and Infants With (Suspected) COVID-19, 8.1.2. Procedures in the Event of (Suspected) COVID-19	Clarification of the procedure to follow if a participant tests positive for SARS-CoV-2 infection.	Clarification
1.3 Schedule of Activities 8.1.2 Procedures in the Event of (Suspected) COVID-19	It has been clarified closure of a COVID-19 episode should occur at the last study visit. If the episode is ongoing, it should be marked as such in the eCRF and followed by the investigator and the outcome recorded in the participant's medical chart.	Clarification
Throughout the protocol	Minor grammatical, formatting, or spelling changes were made.	Minor errors were noted

Amendment 4 (13 December 2021)

Overall Rationale for the Amendment: The main purpose of this amendment is to:

- Allow the inclusion of participants previously vaccinated with a COVID-19 vaccine, who have completed a primary vaccine regimen at least 6 months prior to receiving the study vaccine into the study. These participants will be offered a single booster vaccination of the Ad26.COV2.S at the 5×10^{10} vp dose level. Note: throughout the protocol, previously vaccinated participants refer to participants previously vaccinated with a COVID-19 vaccine.

- Participants who were vaccine naïve at study entry, will be offered an (optional) booster vaccination of Ad26.COV2.S at the 5×10^{10} vp dose level. This booster should be administered after delivery. The booster vaccination will be administered not earlier than 2 months after completion of the participant's Ad26.COV2.S vaccination in the study. The booster vaccination and the booster follow-up visit should not extend the study duration.

- If, after review of the Day 8 safety data of the Sentinels and Safety Cohort by the IDMC, the safety and reactogenicity profile is considered acceptable, the remaining 375

participants will receive 1 dose of Ad26.COV2.S at the 5×10^{10} vp dose at study entry. The arm of 2 doses of Ad26.COV2.S at the 2.5×10^{10} vp dose level (N 75) will be removed from the main study.

- If the safety and reactogenicity profile is not considered acceptable, the remaining 375 participants will continue to receive 2 doses of Ad26.COV2.S at the 2.5×10^{10} vp. This remains unchanged.

Rationale:

- Rationale for including participants previously vaccinated with a COVID-19 vaccine and provide a single booster and rationale for offering participants who were vaccine naïve at study entry a booster:

  On October 20, 2021, the FDA authorized a single booster dose of the Janssen (Johnson and Johnson) COVID-19 Vaccine administered at least 2 months after completion of the single dose primary regimen to individuals 18 years of age and older. Also on October 20, 2021, the FDA authorized the use of a heterologous (or "mix and match") booster dose for currently available (ie, FDA authorized or approved) COVID-19 vaccines.

  A single dose of Ad26.COV2.S vaccine is immunogenic and highly efficacious against severe COVID-19 disease and COVID-19 related hospitalization and death. While protection against variants of concern (such as the Beta and Mu variants in study COV3001 and the Delta variant in the Sisonke study [Gray 2021]) remains high against severe disease, hospitalization, and death, this protection is lower against, eg, the Gamma variant compared to the reference Wuhan strain.

  A second dose of Ad26.COV2.S results in increases of humoral immune responses, and was also shown to result in better protection against COVID-19, as shown in the primary analysis of study COV3009 (Briefing Document 2021). Humoral immune responses (including binding and neutralizing antibodies) have been shown to correlate with protection against COVID-19 (Earle 20201; Khoury 2021; Goldblatt 2021). Vaccination recommendation bodies (CDC 2021) have recently advised to give a booster vaccination (FDA 2019; FDA 2021).

  Should participants decide to receive an alternative vaccine outside the study participants will be encouraged to remain in the study.

- Rationale for removing the 2-dose schedule of 2.5×10^{10} vp dose level (N 75) from the main study:

  The 5×10^{10} vp dose level was used in Janssen's Phase 3 efficacy studies and has shown to elicit strong immunogenicity with an acceptable safety profile. Therefore, if safety and reactogenicity profile is considered acceptable, the lower dose will not be further explored.

RATIONALE:

These and other changes made to the clinical protocol of study VAC31518COV2004 as part of Protocol Amendment 4 are listed below, including the rationale for each change and a list of all applicable sections. Changes made as part of Protocol Amendments 1 to 3 are listed in Section 10.14.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.2 Schema 1.3.1 Adult Participants (Sentinels, Safety and Group 4): 1-dose Schedule 1.3.1.1 Vaccine Naïve Adult Participants (Sentinel, Safety and Group 4): Booster Vaccination Visits 1.3.1.2 Vaccine Naïve Adult Participants (Sentinels, Safety and Group 4): Booster Vaccination Schedule 1.3.2 Adult Participants: Group 1-3: previously vaccinated participants: dose 1 Schedule 2.3.1 Risks Related to Study Participation 2.3.2 Benefits of Study Participation 1.3 OBJECTIVES AND ENDPOINTS 1.4 STUDY DESIGN 1.4.2 Justification for the Regimen and Dose 5 STUDY POPULATION 5.5 Criteria for Temporarily Delaying Administration of Study Vaccination 6.1 Study Vaccinations Administered 6.2 Booster Vaccination 81.8 STUDY ASSESSMENTS AND PROCEDURES 9 STATISTICAL CONSIDERATIONS 10.2 Appendix 2: Clinical Laboratory Tests	Text updated to indicate that a booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp will be offered to ongoing, consenting participants who were vaccine naïve at study entry, and have completed pregnancy. The booster vaccination will be administered not earlier than 2 months after the participant's Ad26.COV2.S vaccination in the study. Text updated to indicate that following IDMC review, the remaining 375 participants will receive either 1 dose of Ad26.COV2.S at 5×10^{10} vp if the safety profile is acceptable or 2 doses of Ad26.COV2.S at 2.5×10^{10} vp if the safety profile is not acceptable irrespective of gestational age. Adult Participants dose 1 schedule updated. Post booster safety and immunogenicity will be assessed 28 days post booster. Thereafter, participants will be followed per the 1-dose schedule of activities until the end of the study (approximately 12 months postpartum). Previously vaccinated pregnant women are now included in the study, thus inclusion and reference to previously vaccinated women was added. Information regarding the booster for previously vaccinated participants (Groups 1-3) and vaccine naïve participants (Group 4) was added. Removed information regarding innate and inflammatory subsets, as these will no longer be included in this study. Updated the text to indicate that 60 mL of blood will be collected from participants who receive the booster vaccination. All applicable sections have been updated accordingly.	See overall rationale for amendment.
2 INTRODUCTION	Updates made to sections 2.1 and 2.2.	Update.

Section Number and Name	Description of Change	Brief Rationale
1.3.5 Procedures for Adult Participants With (Suspected) COVID-19 1.3.6 Procedures for Neonates and Infants With (Suspected) COVID-19 1.3 OBJECTIVES AND ENDPOINTS 8.1.2.2 Procedures for Neonate and Infant Participants in Case of Signs and Symptoms of COVID-19	References to the collection of saliva samples in the modality of collection in the event of a (suspected) COVID-19 infection were deleted from the protocol.	Correction.
2.3.1 Risks Related to Study Participation	Information regarding the risks related to the study participation has been updated (eg, information with regard to TTS and GBS).	Alignment across Ad26.COV2.S protocols.
1.3.1 Adult Participants (Sentinels, Safety and Group 4): 1-dose Schedule 1.1 Synopsis	In the Schedule of Activities, it was erroneously indicated that the footnote: "To be repeated pre-vaccination if the screening test was done more than 4 days before Day 1" was applicable to the *Obstetric Ultrasound* assessment performed at Day 1 (vaccination day). This footnote is applicable to the nasal swab sample taken on Day 1 only. Visits 3 and 4 were removed from the table. Wording regarding the PBMC subset being specific to vaccine naïve participants added to the SoA. Removed information regarding participants in the innate and pro-inflammatory subsets, as these will no longer be included in this study.	Correction.
1.1 Synopsis 1.4 STUDY DESIGN 3 OBJECTIVES AND ENDPOINTS 8.4 Virology Assessments	The possibility of performing sequencing if a sample is available has been added to the protocol.	To identify SARS-CoV-2 variants in available samples.
1.1 Synopsis 1.1.2 Schema 1.4 STUDY DESIGN 1.4.2 Justification for the Regimen and Dose	The staggered enrollment of participants, safety reviews and consequences of the safety review outcome on the vaccine regimen that will be studied has been clarified.	Clarification.
1.1 Synopsis 9.5.2 Interim Analysis 2	The timing of the interim analyses and the data in scope of the analyses has been clarified.	Clarification.
1.3.5 Procedures for Adult Participants With (Suspected) COVID-19 1.3.6 Procedures for Neonates and Infants With (Suspected) COVID-19 8.1.2.1 Procedures for Adult Participants in Case of Signs and Symptoms of COVID-19 8.1.2.2 Procedures for Neonate and Infant Participants in Case of Signs and Symptoms of COVID-19	Clarification was added that for participants with a positive test result for SARS-CoV-2 infection, a study visit will be conducted 28 days after symptom onset (ie, at the COVID-19 Day 29 visit) to assess the clinical course of the infection.	Clarification.
1.3.7 Participants with a Suspected AESI - Pregnant Participants	The naming 'Janssen Adjudication Committee' was corrected to 'AESI Adjudication	Consistency throughout protocol.

Section Number and Name	Description of Change	Brief Rationale
	Committee', in alignment with other places in the protocol.	
1.3.3 Adult Participants (Group 1-4, if applicable): 2-dose Schedule	It has been clarified that the Day 85 visit of the 2-dose schedule will be a phone call and, hence, there will not be an assessment of vital signs or physical examination at that time.	Correction.
8 STUDY ASSESSMENTS AND PROCEDURES 4.1 Overall Design	Blood volumes mentioned in Section 8, Table 1, and throughout the protocol have been corrected to reflect the correct blood volumes taken for the different participant (sub) groups. Added information (Table 1 and throughout) regarding vaccine-related biomarkers (whole peripheral blood) PAX gene® and the booster immunogenicity samples. Added information indicating that PBMCs will be collected for analysis of cellular immunogenicity from a subset (N=50) of adult participants who were vaccine naïve at study entry.	Correction.
1.3.5 Procedures for Adult Participants With (Suspected) COVID-19 1.3.6 Procedures for Neonates and Infants With (Suspected) COVID-19 8 STUDY ASSESSMENTS AND PROCEDURES 8.1.1 Immunogenicity Assessments 8.1.2.1 Procedures for Adult Participants in Case of Signs and Symptoms of COVID-19 8.1.2.2 Procedures for Neonate and Infant Participants in Case of Signs and Symptoms of COVID-19 8.6 Biomarkers	Inconsistencies between the Schedule of Activities for participants with suspected COVID-19 (both the SoAs for adults and the SoAs for neonates/infants) and the section outlining the procedures to be performed in case of suspected COVID-19 have been aligned. Removed information regarding participants in the innate and pro-inflammatory subsets from Table 1 and Table 2, and from Sections 8.1.1 and 8.6 and in the text, as this subset will no longer be included in this study.	Corrections
1.3.3 Adult Participants (Group 1-4, if applicable): 2-dose Schedule	It has been clarified that blood sampling at Day 57 (Visit 8 of the 2-dose schedule) must occur pre-vaccination.	Clarification.
3 OBJECTIVES AND ENDPOINTS 1.1 Synopsis	The following exploratory objective, which was erroneously omitted for the objectives for the adult participants but was included for the neonates/infants, has been added: *To assess SARS CoV 2 viral load in SARS CoV 2 infected participants during a confirmed COVID 19 episode.* Added wording regarding vaccine naïve participants and booster assessments for specific endpoints.	Correction.
1.3.3 Adult Participants (Group 1-4, if applicable): 2-dose Schedule1.3.3 Adult Participants (Group 1-4, if applicable): 2-dose Schedule	Footnote 'a' in the 2-dose Schedule of Activities has been revised: *In case of premature birth before vaccination 2, the need for a second dose should be evaluated by the investigator and the mother. In case no second vaccination is*	Correction.

Section Number and Name	Description of Change	Brief Rationale
	received, the assessments of Visit 15 and the 12 months follow up phase will be initiated. In case a second vaccination will be received, all Visits (except Pregnancy Week 36 Follow up phone call) will have to be completed.	
1.3.7 Participants with a Suspected AESI - Pregnant Participants 8.2.3.1 Hematology Clinical Laboratory Assessments	It is clarified that also in the event of thrombocytopenia, laboratory assessments (to be performed locally) are required to facilitate diagnosis and determine treatment options, including but not limited to platelet count and anti-PF4 tests. It is clarified that all local laboratory results need to be encoded in the eCRF, including platelet counts. Low platelet counts are to be recorded as suspected AESI (thrombocytopenia).	Clarification.
9 STATISTICAL CONSIDERATIONS 9.5 Planned Analyses 1.1 Synopsis	Tables 3 and 5 were updated. The text throughout was updated to indicate that analyses will be performed by group, and overall. Added text indicating that the SAP will detail the 2 planned interim analyses, the primary analysis and the final analysis, and that additional unplanned interim analyses may be performed for safety and/or immunogenicity to facilitate decision making and for regulatory purposes. Added information in the Interim Analysis 2 regarding the outcomes if the safety profile in the Sentinel and Safety Cohorts is considered acceptable or not, per IDMC recommendation. Updated the Final Analysis to indicate that it will include safety and immunogenicity data.	Clarification.
11 REFERENCES	The reference to the Brighton Collaboration case definition of thrombotic events and thrombocytopenia was updated. Additional references added to the list.	Update.
1.3.1 Adult Participants (Sentinels, Safety and Group 4): 1-dose Schedule 1.3.3 Adult Participants (Group 1-4, if applicable): 2-dose Schedule 1.1 Synopsis	The SoAs were updated to present the recording of AEs more accurately.	Clarification.
1.1 Synopsis	It has been clarified that serological response to vaccination could also be measured by an assay which is equivalent to ELISA.	Clarification.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.3.1 Adult Participants (Sentinels, Safety and Group 4): 1-dose Schedule 1.3.3 Adult Participants (Group 1-4, if applicable): 2-dose Schedule 1.4 STUDY DESIGN 1.6.3 Measures to Minimize Bias: 1.2 Schema 6.5 Dose Modification	It has been clarified that the Day 4 safety review following vaccination of the Sentinels was performed by the sponsor's SRP and discussed with the investigator, and that at the time of writing of this amendment, this review was completed and the safety and reactogenicity of the single dose of Ad26.COV2.S at 5×10^{10} vp was found to be acceptable. Hence, enrollment of the Safety Cohort was initiated and participants in this cohort will receive a single dose of Ad26.COV2.S at 5×10^{10} vp. Recruitment of previously vaccinated and vaccine naïve participants has been updated to the following groups: Comirnaty (Pfizer-BioNTech) or SpikeVax (Moderna) (Group1), Ad26.COV2.S (Janssen)(Group 2), other COVID-19 vaccines (Group 3), and vaccine naïve participants (Group 4).	Clarification and update.
1.1 Synopsis 1.4 STUDY DESIGN 1.3.1 Adult Participants (Sentinels, Safety and Group 4): 1-dose Schedule 1.3.2 Adult Participants: Group 1-3: previously vaccinated participants: dose 1 Schedule 1.3.3 Adult Participants (Group 1-4, if applicable): 2-dose Schedule	The text has been updated to indicate two potential outcomes following IDMC review depending on whether the safety profile of 1 dose of Ad26.COV2.S at 5×10^{10} is acceptable or not. If acceptable, the remaining 375 participants will be enrolled and randomized to receive 1 dose of Ad26.COV2.S at 5×10^{10} vp. If not acceptable, the remaining 375 participants will receive 2 doses of Ad26.COV2.S at 2.5×10^{10} vp administered with a 56 day interval. Previously vaccinated participants (Group 1-3) should have completed a primary vaccine regimen at least 6 months prior to receiving the study vaccine into the study. In addition, a row for the randomization of participants has been added to the SoAs.	Correction.
1.1 Synopsis 1.4 STUDY DESIGN	It is clarified that the Day 8 safety review by the IDMC will not only include the Day 8 data from the Safety Cohort but also from the Sentinels.	Clarification.
5.1 Inclusion Criteria 5.2 Exclusion Criteria	Throughout the in- and exclusion criteria, it has been indicated if changes were made to the criteria through one of the previous (and current) amendments. Other inclusion and exclusion criteria were amended in this protocol amendment Removed exclusion criteria regarding participants previously receiving coronavirus vaccine.	Clarification.

Section Number and Name	Description of Change	Brief Rationale
6.9 Study Vaccination Pausing Rules	Updated the list of events that will lead to a pause in further study vaccination. Added death of an adult participant, considered related to study vaccine or if the causal relationship to the study vaccine cannot be excluded.	Update.
1.1 Synopsis 1.3.1 Adult Participants (Sentinels, Safety and Group 4): 1-dose Schedule 1.3.3 Adult Participants (Group 1-4, if applicable): 2-dose Schedule 1.3.4 Neonate/Infants Schedule 1.4 STUDY DESIGN	Language with regard to the timing of the recording of SAEs, MAAEs, AEs leading to discontinuation has been aligned across sections. Body temperature added to Neonate/Infants Schedule.	Clarification.
1.1.1 Synopsis 9.3 Populations for Analysis Sets	It has been added that the PPI-NVN analysis set will also exclude neonates/infants with major protocol deviations that are expected to impact the immunogenicity outcomes.	Correction.
1.1 Synopsis 1.3 Schedules of Activities 8 STUDY ASSESSMENTS AND PROCEDURES 9 STATISTICAL CONSIDERATIONS	Text regarding the collection of colostrum and breast milk added to the Schedule of Activities and updated in the remaining sections listed.	Clarification.
10.9.1 Appendix 9A: CDC Websitea Underlying Medical Conditions for Adults 10.13 Appendix 13: Visit guidance in case of pregnancy termination before the completion of the applicable Study Period visits	Updated the list of underlying medical conditions that adults are more likely to get severely ill from COVID-19 in Appendix 9A. Updated the visit guidance in case of pregnancy termination before the completion of Study Period Visits in Appendix 10.13.	
Throughout the protocol	Throughout the protocol (including the synopsis), sections/paragraphs, have been moved to the most appropriate location and redundant sections have been deleted for clarity purposes.	Clarification.
Throughout the protocol	Minor errors and inconsistencies were corrected, and minor clarifications were added throughout the protocol.	Correction of minor errors and inconsistencies. Addition of minor clarifications.
Throughout the protocol	The naming ‘Janssen Adjudication Committee’ was corrected to ‘AESI Adjudication Committee’, in alignment with other places in the protocol.	Consistency throughout protocol.

Amendment 3 (02 July 2021)

Overall Rationale for the Amendment: The main purpose of this amendment is to update the safety procedures to include TTS as an AESI, based on spontaneous reports of extremely rare events of TTS observed following administration of the Ad26.COV2.S vaccine under EUA in the US that suggest that there may be an increased risk of a thrombotic event combined with thrombocytopenia associated with the vaccine.

The study design has been amended so that a larger cohort of participants (N = 325) receive the selected vaccine regimen for adults of a single dose of Ad26.COV2.S at 5×10^{10} vp, with a smaller cohort of participants (N = 75) receiving 2-doses of Ad26.COV2.S at 2.5×10^{10} vp. The aim is to evaluate a dosing schedule that has the potential for less reactogenicity, but at least comparable or potentially greater immunogenicity. Enrollment will be staggered with the safety and reactogenicity evaluated in a group of Sentinels (n=5) followed by a larger Safety Cohort (n=20), before proceeding to full enrollment. If the safety profile of Ad26.COV2.S at 5×10^{10} vp is not acceptable in pregnant women, then the remaining 375 participants will be administered 2-doses at the lower dose of 2.5×10^{10} vp at a 56-day interval.

Section Number and Name	Description of Change	Brief Rationale
1.1. Synopsis, 1.3.1. Adult Participants: 1-dose Schedule, 1.3.2. Adult Participants: 2-dose Schedule, 1.3.6. Participants with a Suspected AESI - Pregnant Participants, 2.3.1. Risks Related to Study Participation 2.3.3. Benefit-Risk Assessment for Study Participation, 3. OBJECTIVES AND ENDPOINTS, 4.1. Overall Design, 6.8. Prestudy and Concomitant Therapy, 6.9. Study Vaccination Pausing Rules, 8.1.1. Immunogenicity Assessments, 8.2.3.1. Hematology Clinical Laboratory Assessments, 8.3. Adverse Events, Serious Adverse Events, Adverse Events of Special Interest, and Other Safety Reporting, 8.3.1. Time Period and Frequency for Collecting Information Relating to Adverse Events, Adverse Events of Special Interest, Serious Adverse Events and Medically-Attended Adverse Events, 8.3.2. Method of Detecting Adverse Events, Adverse	Thrombosis with thrombocytopenia syndrome (TTS) will be considered an AESI. Follow-up assessments will be performed in the event of a suspected AESI. Participants will be asked daily via the eCOA if they have experienced any health concerns (including new onset of symptoms associated with TTS) within the 30 day time period post-vaccination, and if so, participants will be advised to contact the study center.	Emerging data following use of the Ad26.COV2.S vaccine under EUA in the US suggest an increased risk of TTS, with onset of symptoms approximately 1-3 weeks after vaccination. Therefore, additional reporting and data collection procedures are implemented to follow-up thrombotic events and thrombocytopenia and identify cases of TTS.

Section Number and Name	Description of Change	Brief Rationale
Events of Special Interest, Serious Adverse Events and Medically-attended Adverse Events, 8.3.3. Follow-up of Adverse Events, Serious Adverse Events, Adverse Events of Special Interest and Medically-Attended Adverse Events, 8.3.5. Adverse Events of Special Interest, 8.3.5.1. Thrombosis with Thrombocytopenia Syndrome (TTS), 9.4.2. Primary/Secondary Endpoint(s), 10.2. Appendix 2: Clinical Laboratory Tests 10.3.6. Safety Monitoring Committees Structure, 10.4. Appendix 4: Adverse Events, Serious Adverse Events, Adverse Events of Special Interest, Product Quality Complaints, and Other Safety Reporting: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting, 10.4.5. Procedures, 10.11. Appendix 11: Thrombosis with Thrombocytopenia (TTS) AESI Form, 10.12. Appendix 12: Thrombotic Events to be Reported as Suspected AESIs, 11. REFERENCES		
1.1. Synopsis, 1.3.6. Participants with a Suspected AESI - Pregnant Participants, 3. OBJECTIVES AND ENDPOINTS, 8.2.3. Clinical Safety Laboratory Assessments, 8.3.5. Adverse Events of Special Interest, 10.2. Appendix 2: Clinical Laboratory Tests	Serum and plasma samples obtained at baseline and post-vaccination will be used for retrospective analysis of coagulation-related parameters. In the event of an AESI, coagulant factors that are thought to be associated with TTS, including anti-PF4 antibodies, lupus anticoagulant, anti-β2 glycoprotein, D-dimer levels and anti-cardiolipin will be assessed.	This was added to fully evaluate coagulopathy-related parameters per current medical practice

Section Number and Name	Description of Change	Brief Rationale
1.1. Synopsis, 1.3.1. Adult Participants: 1-dose Schedule, 1.3.2. Adult Participants: 2-dose Schedule, 3. OBJECTIVES AND ENDPOINTS, 8.3.1. Time Period and Frequency for Collecting Information Relating to Adverse Events, Adverse Events of Special Interest, Serious Adverse Events and Medically-Attended Adverse Events,	For the primary endpoints: Adverse evets, including solicited local and systemic AEs will be monitored for 7 days after (each) vaccination, or until resolution. Additional safety assessment at 14 days post-vaccination (1-dose schedule and first dose of 2-dose schedule). Timepoints were specified for the assessment of humoral immune response 28 days after the first vaccination and 14 days after the second vaccination. For the 2-dose schedule, the Day 85 (28 days post-vaccination 2) visit will become a phone call visit for safety-follow-up.	Update timepoints for assessment of humoral responses post-vaccination 1 and post-vaccination 2.
2. INTRODUCTION, 2.1. Study Rationale	Text updated to include data from the Reproduction and Developmental Toxicity Study for Ad26.COV2.S.	Update relevant background information to support study rationale.
1.1. Synopsis, 1.2. Schema, 2. INTRODUCTION, 3. OBJECTIVES AND ENDPOINTS, 4.1. Overall Design, 4.3. Justification for Dose, 6.1. Study Vaccinations Administered, 6.3. Measures to Minimize Bias: Randomization and Blinding, 9.2.1. Immunogenicity, 9.2.2. Safety, 9.5.2. Interim Analysis 2 9.6. Independent Data Monitoring Committee.	The study design has been amended so that a larger cohort of participants (N = 325) receive the selected vaccine regimen for adults of a single dose of Ad26.COV2.S at 5×10^{10} vp, with a smaller cohort of participants (N = 75) receiving 2-doses of Ad26.COV2.S at 2.5×10^{10} vp. If the safety profile of Ad26.COV2.S at 5×10^{10} vp is not acceptable, then a 2-dose schedule of Ad26.COV2.S at a dose of 2.5×10^{10} vp will be administered to all remaining participants (N=375) at an interval of 56 days.	To mitigate safety concerns relating to potential reactogenicity associated with the use of a 2-dose schedule of Ad26.COV2.S at 5×10^{10} vp in pregnant women, all participants will receive a single dose of Ad26.COV2.S at 5×10^{10} vp, unless the safety profile in the Sentinels and Safety Cohort is not acceptable, in which case all participants will receive a 2-dose schedule of Ad26.COV2.S at 2.5×10^{10} vp.
7.1. Discontinuation of Study Vaccination	The following text was added: Participants who have previously experienced an event of TTS, including CVST, or HIT.	Update To ensure that a participant's study vaccination must be discontinued if the participant has experienced an event of TTS, including CVST or HIT after receiving the first dose of vaccine (applicable to the 2-dose schedule).
7.1. Discontinuation of Study Vaccination	The following text was added: Participants who have previously experienced an event of CLS.	Update To ensure that a participant's study vaccination must be discontinued if the participant has experienced an event of CLS after receiving the first dose of vaccine (applicable to the 2-dose schedule).

Section Number and Name	Description of Change	Brief Rationale
1.1. Synopsis, 4.2. Scientific Rationale for Study Design, 5.2. Exclusion Criteria	Exclusion criterium added: Participant has a history of CLS.	Update To ensure that participants with a risk of CLS are not included in the clinical study
1.1. Synopsis, 4.2. Scientific Rationale for Study Design, 5.2. Exclusion Criteria	Exclusion criterium added: TTS, including CVST, or HIT.	Update To ensure that a participant that has a history of TTS, including CVST, or HIT are not included in the clinical study.
5.2. Exclusion Criteria	7. Exclusion criterion relating to a participant that is an employee of the investigator or study site, with direct involvement in the study, is reinstated.	Criterion reinstated for consistency across studies.
1.1. Synopsis, 1.3.1. Adult Participants: 1-dose Schedule, 1.3.2. Adult Participants: 2-dose Schedule 3. OBJECTIVES AND ENDPOINTS, 4.1. Overall Design, 8.1.1. Immunogenicity Assessments 8.6. Biomarkers	The exploratory objectives and endpoints were updated for adults and neonates. These updates are reflected in the Table of Summary of Humoral and Cellular Immunogenicity Assays.	Update to include additional exploratory objectives and endpoints in line with other study protocols within the clinical program.
1.1. Synopsis, 1.3.1. Adult Participants: 1-dose Schedule, 1.3.2. Adult Participants: 2-dose Schedule, 3. OBJECTIVES AND ENDPOINTS, 8.1.1. Immunogenicity Assessments, 8.5. Genetics and Pharmacogenomics	The text was updated to include evaluation of innate, pro-inflammatory and other relevant pathway responses to Ad26.COV2.S vaccination in a subset of participants (N=25 from each cohort) at selected timepoints: - Analysis of mRNA expression levels of vaccine-induced innate responses, including inflammatory and coagulation-related mediators. - Analysis of cytokines, chemokines, and other protein-or lipid mediators of the innate immune response.	Update to include additional analysis of immunological pathways associated with Ad26.COV2.S vaccination.
1.1. Synopsis, 3. OBJECTIVES AND ENDPOINTS,	The exploratory endpoint of 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 was added.	Update to include SARS-CoV-2 viral load as an exploratory endpoints.
1.3.1. Adult Participants: 1-dose Schedule, 1.3.2. Adult Participants: 2-dose Schedule, 1.3.3. Neonate/Infants Schedule, 8.2.1. Physical Examinations	Text updated for clarification of procedures that are related to the pregnant mother and neonate, including: Collection of breast milk and colostrum; Safety assessments: Obstetric examination (will not be performed at PP1 visit), Physical examination; and medical and obstetric history.	Clarification relating to procedures.

Section Number and Name	Description of Change	Brief Rationale
1.3.4. Procedures for Adult Participants With (Suspected) COVID-19, 1.3.5. Procedures for Neonates and Infants With (Suspected) COVID-19, 8.1.2. Procedures in the Event of (Suspected) COVID-19	Clarification in the footnotes that central laboratory testing for SARS-CoV-2 infection will be done only after a local positive test. Additional information added to support procedures in case a participant cannot attend the study site due to COVID-19. Clarification that in the event that sites cannot support collection of the nasal swab and saliva samples from the participants home, that HCP visits will be needed.	Clarification in relation to testing for SARS-CoV-2 in the event a local test is positive. Clarification of procedures in case of travel restrictions due to the ongoing COVID-19 pandemic Alignment with other Phase 3 protocols.
2.3.1. Risks Related to Study Participation	Text has been added clarifying that genetic test results obtained during the course of this study are for exploratory research purposes and will not be provided to the participant.	Protect the participant from disclosure of personal genetic information gathered for exploratory research purposes only.
1.3.1. Adult Participants: 1-dose Schedule; 1.3.2. Adult Participants: 2-dose Schedule, 8.1.1. Immunogenicity Assessments,	Blood sampling timepoints and total blood volumes to be drawn were updated to align with other changes described in this table.	Blood sampling timepoints and total blood volumes updated in line with updates to study design.
9.5. Interim Analyses	Interim Analysis 3 (optional) deleted.	Not required
Throughout the protocol	Minor grammatical, formatting, or spelling changes were made.	Minor errors were noted

Amendment 2 (19 March 2021)

Overall Rationale for the Amendment: The main purpose of this amendment is to update the study design based on health authority feedback. The design was updated from a 2- dose regimen to a 1- or 2- dose regimen, thereby including the vaccination regimen with currently available supportive efficacy data and authorized under EUA (US)/Conditional Marketing Authorization (EU). In addition, halting rules were added for pregnancy-related complications; an additional interim analysis was added for a potential total of 3 interim analyses; exclusion of participants who are seropositive at baseline was removed; assessment of neurodevelopmental status of neonates/infants born of participants that received the vaccine during the 2nd and/or 3rd trimester of pregnancy was added; viral neutralizing antibody assay was added as a secondary endpoint; plasma collection at different timepoints was added to allow adequate evaluation in case of occurrence of a thromboembolic event.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis; 1.2 Schema; 1.3.1Adult Participants: 1-dose Schedule; 3 OBJECTIVES AND ENDPOINTS; 4.1 Overall Design; 6.1 Study Vaccinations Administered; 6.5 Dose Modification	Inclusion of a second group of participants who will only receive 1 dose of the vaccine at 5×10^{10} vp,	Inclusion of the vaccination regimen with currently available supportive efficacy data and authorized under EUA (US)/Conditional Marketing Authorization (EU)
1.1 Synopsis; 4.1 Overall Design; 6.5 Dose Modification; 9.6 Independent Data Monitoring Committee	Text was added to clarify that the fallback regimen for unacceptable tolerability is a 2-dose schedule of Ad26.COV2.S at a dose level of 2.5×10^{10} vp, and that if this regimen is used, Sentinels and participants in the Safety Cohort who were randomized to receive Ad26.COV2.S at a dose level 5×10^{10} vp will not receive a second vaccination.	Clarification
1.1 Synopsis; 1.3 Schedules of Activities; 6.3 Measures to Minimize Bias: Randomization and Blinding	Text was revised from no randomization to stratified randomization of participants to the 1- or 2-dose regimen.	Inclusion of a second group of participants who will only receive 1 dose of the vaccine at 5×10^{10} vp, and inclusion of the pregnancy stage stratification factor
1.1 Synopsis; 3 OBJECTIVES AND ENDPOINTS 9.2.1 Immunogenicity; 9.4.3 Exploratory Endpoint(s)	Secondary objectives were grouped by Adult Participants and Neonates/Infants. For adult participants, humoral immune response at 28 days post-vaccination was moved from primary to secondary objective; humoral immune response based on ELISA and VNA titers were made Secondary objectives.	To improve readability/clarity. Based on health authority feedback, assessment of neutralizing antibody responses 28 days after each vaccination in all adult participants and their neonates (cord blood) is a secondary objective; assessment at other timepoints is exploratory.
1.1 Synopsis; 3 OBJECTIVES AND ENDPOINTS; 9.2.1 Immunogenicity; 10.1 Appendix 1: Abbreviations	Reference to “pseudoviron” and “wild-type” VNA was deleted.	The type of VNA to be used will be determined based on assay performance.

Section Number and Name	Description of Change	Brief Rationale
2 INTRODUCTION	Epidemiology data were updated for SARS-CoV-2 and toxicology data were updated for Ad26.COV2.S.	Update
5.5 Criteria for Temporarily Delaying Administration of Study Vaccination; 7.2 Delay or Discontinuation of Vaccination due to Pregnancy-Specific Complications (added)	Addition of halting rules specific for pregnancy complications.	To ensure the safety of participants with pregnancy-related complications
9.5.1 Interim Analysis 1; 9.5.2 Interim Analysis 2; 9.5.3 Interim Analysis 3(added)	Text for Interim Analysis 1 was updated from "will take place" to "may take place." Interim Analysis 2 was updated to occur when all 400 participants have completed the Day 29 visit (28 days post-dose 1) and to include immunogenicity data. Addition of Interim Analysis 3, which may take place when all 400 participants have completed the Day 85 visit (28 days post Visit 5).	Interim Analysis 1 was made optional. To evaluate safety and immunogenicity data periodically to be able to inform Health Authorities
10.1 Appendix 1: Abbreviations; 10.3.6 Safety Monitoring Committees Structure	Deletion of the statistical support group (SSG).	A separate SSG is not needed in the context of an open-label study
5.1 Inclusion Criteria	Inclusion criterion 10: A footnote was added to indicate that participants with visual impairment are eligible and may have caregiver assistance in completing the eCOA questionnaires.	Clarification
5.2 Exclusion Criteria	Exclusion criterion 17 was deleted.	There is no need to exclude employees or those involved with the study in an uncontrolled open-label design post Emergency Use Authorization/Conditional Marketing Authorization.
5.2 Exclusion Criteria 9.4.1 General Considerations	Exclusion criterion 23 was modified. Exclusion criterion 26 was deleted (redundant in view of the changes to criterion 23). Text was added to indicate that some descriptive analyses may be performed by serostatus at baseline.	Seropositive participants will provide informative safety data on vaccination in this subpopulation of pregnant women and their neonates/infants, especially given the increasing prevalence of seropositive individuals over time. Primary immunogenicity endpoints will be summarized by baseline serostatus to assess the impact of preexisting immunity.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis; 3 OBJECTIVES AND ENDPOINTS; 4.1 Overall Design; 8.1.1 Immunogenicity Assessments; 9.2 Sample Size Determination; 9.4.3 Exploratory Endpoint(s)	Neutralizing antibodies will be collected and assessed in all participants and their neonates/infants as a secondary endpoint; text related to a subset of participants for VNA was deleted from sample size determination.	Current data suggest neutralizing antibody levels may be informative of protection, in addition to binding antibodies. Inclusion of all participants in this analysis will provide more information and allow for stratification between 2nd and 3rd trimesters. Secondary immunogenicity endpoints will be summarized by baseline serostatus to assess the impact of preexisting immunity.
1.1 Synopsis; 1.3.3 Neonate/Infants Schedule; 3 OBJECTIVES AND ENDPOINTS; 8.2.5 Neonatal Assessments; 9.4.3 Exploratory Endpoint(s)	Inclusion of the Ages & Stages Questionnaire, 3rd edition to assess neurodevelopmental status in neonates/infants at 2, 6 and 12 months of age as an exploratory endpoint. Section title was updated.	To evaluate developmental outcomes of the children of study participants
1.3.2 Adult Participants: 2-dose Schedule	Footnote was updated to indicate that the baseline SIC should be completed prior to the first vaccine administration.	If the baseline SIC is not completed, no eCOA scales will be triggered.
1.3.2 Adult Participants: 2-dose Schedule	Blood draw for serologic testing at screening was removed; footnote was added that humoral immunogenicity samples at Day 1, Day 29, Day 85, birth, PP6 and PP12 will be used for baseline and subsequent serology testing. Other footnotes have been clarified.	As we are including seropositives and seronegatives, there is no need for serological samples at screening. The additional timepoints are necessary to evaluate the occurrence of asymptomatic infection. Clarification
1.3.1 Adult Participants: 1-dose Schedule; 1.3.2 Adult Participants: 2-dose Schedule; 8.1.1 Immunogenicity Assessments; 9.4.3 Exploratory Endpoint(s); 10.2 Appendix 2: Clinical Laboratory Tests; 10.3.5 Long-Term Retention of Samples for Additional Future Research	Blood samples (7.5 mL) for plasma will be collected pre-dose on Day 1 and Day 57 (2-dose regimen) and at 7 days and 28 days post-dose 1 and post-dose 2 (if applicable); at delivery and at the postpartum Day 42 follow-up visit, to be stored for future testing to evaluate the impact of any potential thromboembolic events (if applicable) and possibly additional. (Unused plasma samples may be used for immunogenicity testing.) Blood volumes were updated.	Due to concerns of a possible association with adenoviral vector based vaccines on the incidence of thromboembolic events within healthy participants and the increased risk of these thromboembolic events associated with pregnancy.

Section Number and Name	Description of Change	Brief Rationale
1.3.4 Procedures for Adult Participants With (Suspected) COVID-19; 1.3.5 Procedures for Neonates and Infants With (Suspected) COVID-19; 8.1.2.1 Procedures for Adult Participants in Case of Signs and Symptoms of COVID-19; 8.1.2.2 Procedures for Neonate and Infant Participants in Case of Signs and Symptoms of COVID-19	Text was added to clarify the requirements for collection of nasal swabs and SIC, respectively, to close the COVID-19 episode for adult participants. A note was added that if the participant is unable to complete the SIC/PedSIC, the reason for the missing assessment should be recorded in the eCRF.	To reduce burden on participants who continue to test positive after symptom resolution
8.1.2.1 Procedures for Adult Participants in Case of Signs and Symptoms of COVID-19; 8.1.2.2 Procedures for Neonate and Infant Participants in Case of Signs and Symptoms of COVID-19	Clarification was added that COVID-19 Day 29 procedures should be completed even if nasal swabs are pending.	Clarification
4.3 Justification for Dose	Text was added to inform about EUA/ Conditional Marketing Authorization of the single dose regimen of Ad26.COV2.S 5×10^{10} vp.	Updated information
1.1 Synopsis; 3 OBJECTIVES AND ENDPOINTS; 6.8 Prestudy and Concomitant Therapy; 8.3.1Time Period and Frequency for Collecting Information Relating to Adverse Events, Adverse Events of Special Interest, Serious Adverse Events ; 9.2.2 Safety; 9.4.2 Primary/Secondary Endpoint(s)	Text was modified to clarify that AEs leading to discontinuation will be reported for adult participants and neonates/infants throughout the study, not just medically-attended AEs leading to discontinuation.	Clarification
4.4 End of Study Definition	Text was revised to define premature discontinuation as not having completed Visit 12.	Replaced reference to completion of the 2-dose regimen, because not all participants will receive 2 doses
9.4.1 General Considerations	Text was updated that analyses may be performed by pregnancy stage, dose regimen, and/or by baseline serostatus. For neonates/infants, analyses may also be performed by vaccination regimen received by the adult participant during pregnancy.	To align with the randomized 1- or 2-dose study design and enrollment of participants regardless of baseline serostatus
10.5 Appendix 5: Contraceptive Guidance	A footnote was added to hormonal contraception “or per physician preference/local standard of care in a woman who is breastfeeding.”	Introduces flexibility, as hormonal contraception may be different in a breastfeeding participant

Section Number and Name	Description of Change	Brief Rationale
10.2 Appendix 2: Clinical Laboratory Tests	Tables were added to outline tests performed on adult participants and neonates/infants.	Consistency with body text
Throughout the protocol	Minor grammatical, formatting, or spelling changes were made.	Minor errors were noted

Amendment 1 (28 February 2021)

Overall Rationale for the Amendment: Pregnancy is associated with an increased risk of severe illness from COVID-19 and potential adverse birth outcomes. The main rationale for the protocol amendment is to document changes to study design which, if implemented, will ensure that all participants enrolled in this study benefit from the protection conferred by the Ad26.COV2.S vaccine. This is considered an ethical imperative, in view of the rising disease burden associated with the current pandemic. The initial proposal was for a randomized double-blind, placebo-controlled study to evaluate the safety and reactogenicity of 2 dose levels (2.5×10^{10} vp and 5×10^{10} vp) of Ad26.COV2.S versus placebo control, administered as a 1-dose or 2-dose schedule, in adult participants during the 2nd and/or 3rd trimester of pregnancy. The current proposal is for an open-label study to assess the safety and reactogenicity of Ad26.COV2.S, administered at a single dose level of 5×10^{10} vp, as a 2-dose schedule (at a 56-day interval, if feasible) to all study participants during the 2nd and/or 3rd trimester of pregnancy (or postpartum, if necessary). There will be no placebo control group in this study. Interim Analysis will be performed when the first 200 enrolled adult participants have completed the Day 29 visit (28 days post-dose 1) and Day 85 visit (28 days post-dose 2), for the purpose of providing post-dose 1 safety and reactogenicity data for regulatory authorities, if requested to do so.

Section Number and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis; Section 1.2 Schema; Section 1.3.1 Schedule of Activities; Section 3 Objectives and Endpoints; Section 4.1 Overall Design; Section 4.3 Justification for Dose	Change in vaccine dosing regimen: The original proposal was for a randomized double-blind, placebo-controlled study to evaluate the safety and reactogenicity of 2-dose levels (2.5×10^{10} vp and 5×10^{10} vp) of Ad26.COV2.S, administered as a 1 dose or 2-dose schedule. - The current proposal (protocol Amendment 1) is for an open-label study to assess the safety and reactogenicity of Ad26.COV2.S, administered at a single dose level of 5×10^{10} vp, as a 2-dose schedule (at a 56-day interval, if feasible) to all study participants during the 2nd and/or 3rd trimester of pregnancy (or postpartum, if necessary). There will be no placebo control group. The Ad26.COV2.S (2.5×10^{10} vp) dose is not retained in this protocol amendment as the Ad26.COV2.S (5×10^{10} vp) dose showed an acceptable reactogenicity profile in adults 18 years and older.	- To ensure that all participants enrolled in the study are offered the Ad26.COV2.S vaccine at the selected dose of 5×10^{10} vp, using a 2-dose schedule. - To ensure that there is flexibility built into the dosing regimen, which will allow for the second dose of vaccine to be administered postpartum, if necessary.
Section 1.1 Synopsis; Section 4.1 Overall Design; Section 5.1 Inclusion Criteria; Section 9.2 Sample Size Determination	The age range of adult participants enrolled has been extended to ≥ 18 years to ≤45 years of age.	- The age range at which adult participants are recruited has been extended to reflect an increase in pregnancies in older women within

Section Number and Name	Description of Change	Brief Rationale
		the age range of ≥40 years and ≤45 years.
Section 1.1 Synopsis; Section 3 Objectives and Endpoints; Section 4.1 Overall Design; Section 5.1 Inclusion Criteria; Section 6.1 Study Vaccination Administered; Section 9.4.2 Primary/Secondary Endpoints	The gestational age at enrollment has been extended from Weeks ≥20 to ≤36, with enrollment stratified by pregnancy stage at time of randomization to Weeks ≥16 to ≤38 (2nd or 3rd trimester of pregnancy), with no restriction placed on the number of participants, based on age of gestation (within the given range), or on the dosing regimen (ie, 1-dose or 2-dose based on age of gestation). All participants will receive 2-doses of Ad26.COV2.S at the 5×10^{10} vp dose level, irrespective of gestational age. Depending on the stage of pregnancy at the time of enrollment, both the first and second dose of vaccine should be administered during pregnancy, if feasible, with the first dose of vaccination (Day 1) administered following the screening period. The 2nd dose of vaccine will be administered on Study Day 57. For those participants in later stages of pregnancy (ie, 3rd trimester at enrollment) and in those cases in which it is not feasible to administer both vaccines during pregnancy (eg, premature delivery), the second dose of vaccine can be administered postpartum.	- The gestational age of adult participants at enrollment has been extended to Weeks ≥16 to ≤38 (ie, still within the 2nd or 3rd trimester of pregnancy, but with an extended gestational age range), to reflect the real-world situation and the need to provide protection for pregnant women. - Participants will no longer be stratified by pregnancy stage to receive either a 1-dose or 2-dose schedule based on gestation age at the time of randomization. All participants will receive a 2-dose schedule, with the second dose administered postpartum, if necessary.
Section 1.1 Synopsis; Section 4.1 Overall Design; Section 6.5 Dose Modification; Section 9.6 Independent Data Monitoring Committee	The safety and tolerability of a single dose of the Ad26.COV2.S vaccine at the 5×10^{10} vp dose level in pregnant women, will be assessed in the first instance in 5 sentinels at Day 4 (3 days post-vaccination) and if acceptable, in the larger Safety Cohort (20 participants) at Day 8 (7 days post-vaccination), before proceeding to full enrollment. An IDMC will review the open-label safety and reactogenicity data: Only after positive recommendations from the IDMC following assessment of the Day 8 safety and reactogenicity profile in the Safety Cohort, will the remaining 375 participants be enrolled to received 2 doses of Ad26.COV2.S at 5×10^{10} vp. If the safety profile after 1 dose of Ad26.COV2.S at the 5×10^{10} vp dose in the group of Sentinels and/or in the Safety Cohort is not considered acceptable, all remaining participants will receive 2	To ensure that the safety and reactogenicity of the Ad26.COV2.S vaccine at the 5×10^{10} dose level is monitored and assessed by the Sponsor and the IDMC in a Safety Cohort of 25 pregnant adult participants (5 sentinels initially, and a further 20 participants), before proceeding to full enrollment, with the aim of minimizing exposure in pregnant women in the event of safety concerns.

Section Number and Name	Description of Change	Brief Rationale
	doses of Ad26.COV2.S at the 2.5×10^{10} vp dose level as shown in the figure below. The sequential enrollment is depicted in the figure below. All participants (including sentinels and those assigned to the Safety Cohort) will receive 2-doses of Ad26.COV2.S.	
Section 1.1 Synopsis; Section 1.3.1 Schedule of Activities Section 2.3.3 Benefit-Risk Assessment for Study Participation; Section 4.1 Overall Design; Section 8.3.2 Method of Detecting Adverse Events, Medically-attended Adverse Events, and Serious Adverse Events Information	Sentinels and participants in the Safety Cohort will be vaccinated at least 1 hour apart and will remain under observation at the site for at least 60 minutes to monitor for the presence of any acute reactions and solicited events. Participants in the Sentinel and Safety Cohorts will continue to be closely monitored for 7 days post-vaccination and will be requested to record solicited signs and symptoms in a reactogenicity diary. All other participants will remain under observation at the study site for at least 30 minutes and will be requested to record solicited signs and symptoms in a reactogenicity diary for 7 days post-vaccination.	To ensure that the Safety and reactogenicity is monitored post-vaccination.
Section 6.3 Measures to Minimize Bias: Randomization and Blinding Section 6.6 Continued Access to Study Vaccine After the End of the Study; Section 10.3.6 Safety Monitoring Committee Structure	In the original protocol, participants were randomized to Groups 1-4 (Safety Cohort including Sentinels) with the remaining participants randomized to Groups 5-10 (stratified by number of weeks gestation and study regimen). Based on the current study design, randomization will not be used in this study. Participants will no longer be stratified by pregnancy stage. All participants (including those in the sentinel group and safety cohort) will be assigned in an open-label manner to receive 2 doses of the Ad26.Cov2.S vaccine at a dose level of 5×10^{10} vp. As an open-label study, blinding procedures are no longer applicable. Continued Access to Study Vaccine After the End of the Study: Not applicable.	Clarification that participants will no longer be assigned to separate study groups based on randomization and stratification by pregnancy stage and study site.
Section 1.1 Synopsis; Section 4.1 Overall Design; Section 9.2 Sample Determination	The change from a double-blind, placebo-controlled study to an open-label study has had an impact on sample size calculations, with a reduction in numbers of participants enrolled: Based on the proposed study regimen, a target of 400 adult participants will be enrolled (down	Clarification

Section Number and Name	Description of Change	Brief Rationale
	from 824 adult participants in the original study design).	
Section 9.2.1 Safety (Endpoints); Section 9.3 Populations for Analysis Sets; Section 9.4.2 Primary/Secondary Endpoints Section 9.5 Interim Analyses; Section 9.5.1 Interim Analysis 1; Section 9.5.2 Interim Analysis 2; Section 9.7 Primary Analysis	Statistical Consideration (including sample size, populations for analysis sets, statistical analysis, and safety endpoints) have been updated to reflect the impact of changing from a randomized double-blind, placebo-controlled study to an open-label study. - Interim analysis will now be performed on the first 200 enrolled adult participants, with Interim Analysis 1 performed when the first 200 enrolled adult participants have completed the Day 29 visit (ie, 28 days post-dose 1), and Interim Analysis 2 performed when the first 200 enrolled adult participants have completed the Day 85 visit (ie, 28 days post-dose 2). Primary/Secondary Safety Endpoints: - No formal statistical testing of safety data is planned. Safety data will be analyzed descriptively for adult participants. Safety data for participants who receive the second dose of vaccine postpartum may be provided as a separate descriptive analysis. - Safety endpoints: updated in line with revised definitions for pregnancy outcomes and outcomes in neonates/infants. Clarification that primary analysis of safety and immunogenicity will be performed when all adult participants have completed the visit that takes place "approximately" 42 days postpartum.	Statistical Considerations include Interim Analysis to take place when the first 200 enrolled adult participants have completed the Day 29 visit (28 days post-dose 1) and Day 85 visit (28 days post-dose 2), for the purpose of providing post-dose 1 and post-dose 2 safety and reactogenicity data for regulatory authorities, if requested to do so. Statistical Considerations for analysis of safety data for women who receive the second dose of vaccine postpartum.
Section 5.2 Exclusion Criteria; Section 10.5 Appendix 5: Contraceptive Guidance	Exclusion Criterium 28 added: At the time of consenting, participants should agree to practicing an acceptable effective method of contraception postpartum and agree to remain on such a method of contraception following the birth of the infant until 3 months after administration of the second study vaccine. Participants should follow contraceptive (birth control) measures consistent with local regulations regarding the acceptable methods of contraception	To reflect the enrollment of participants in the later stages of pregnancy (ie, 3rd trimester at enrollment) and those cases in which it is not feasible to administer both vaccines during pregnancy (eg, premature delivery), and the second dose of vaccine can be administered postpartum.

Section Number and Name	Description of Change	Brief Rationale
	Appendix 5: Contraceptive Guidance added.	
Section 5.2. Exclusion Criteria	Exclusion Criterium No. 25 deleted. A participant who is currently working in an occupation with a high-risk of exposure to SARS-CoV-2 infection or considered to be at increased risk to acquire COVID-19 for any other reason, will no longer be excluded from enrollment.	To reflect the fact that pregnant women are unlikely to be working in an occupation where they are at increased risk of exposure to SARS-CoV-2 infection; participants who are at risk due to exposure in the general population should not be excluded from enrollment.
Section 1.3.1 Schedule of Activities	Footnote (bb) added to Schedule of Activities: Urine pregnancy test should be performed at Study Visit 5 (ie, prior to Vaccination 2). This is only applicable to women who are ≥6 weeks postpartum (ie, only applies to women in those cases in which the second dose of vaccine is to be administered after birth).	To reflect the enrollment of participants in the later stages of pregnancy (ie, 3rd trimester at enrollment) and those cases in which it is not feasible to administer both vaccines during pregnancy (eg, premature delivery), and the second dose of vaccine can be administered postpartum (ie, there is a possibility that the participant may become pregnant prior to receipt of the second dose).
Section 1.3.1 Schedule of Activities Section 8.1.1 Immunogenicity Assessments	All participants will be given the option to provide breast milk and colostrum on a voluntary basis (previously obtained from a subset of participants [N=60]). The volume of breast milk is adjusted to approximately 10 mL (previously 5 mL).	To ensure that breast milk and colostrum are provided by participants on an optional basis.
Section 1.3.1 Schedule of Activities; Section 8.1.1 Immunogenicity Assessments	Blood samples for PBMC isolation for assessment of cellular immunity will be obtained from a subset of 60 participants (previously, a subset of 260 participants).	The number of participants from whom blood samples will be collected for isolation of PBMC has been adjusted to reflect the change in study design.
Section 1.3.2 Schedule of Activities; Section 1.3.4 Schedule of Activities; Section 8.1.1 Immunogenicity Assessments	Peripheral blood samples collected for assessment of humoral immunity in neonates/infants has been updated: - Schedule of Activities 1.3.2: Blood samples of 3.5 mL to be collected at Study Visit 4 and Visit 8 (ie, at 2 months and 6 months of age); - Schedule of Activities 1.3.4: Blood samples of 2.5 mL to be collected. Cord blood samples to be collected for assessment of humoral immunity in neonates/infants: Approximately 15 mL (minimum of 10 mL) - Schedule of Activities 1.3.2 and - Table 1. Immunogenicity Assessments	The peripheral blood volumes and cord blood volumes to be collected have been revised.

Section Number and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis; Section 3 Objectives and Endpoints; Section 4.1 Overall Design; Section 9.2.1 Safety (Statistical Considerations); Section 9.4.2 Primary/Secondary Endpoints	Definitions for Pregnancy outcomes and outcomes in neonates/infants updated as follows: - Pregnancy outcomes (including live term birth live preterm birth, stillbirth, and abortion). - Outcomes in neonates and infants (including, normal neonate, term neonate with complications, preterm neonate with 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]).	To align with revised definition used in the electronic Case Report Form (eCRF).
Section 6.9 Study Vaccination Pausing Rules; Section 10.3.6 Safety Monitoring Committee	Text relating to the Safety Monitoring Committee updated. Text relating to procedures to be performed in the event of a study pause added, specifying that "if following appropriate safety review it is deemed appropriate to restart dosing, the sponsor must submit a request to restart dosing with pertinent data to competent authority as a request for a substantial amendment, as required by local regulations or authority request (eg, MHRA). If needed, this will be followed by a substantial amendment of the IB and/or protocol."	Clarification on the procedures to be followed to initiate vaccination in the event that a study vaccination pausing rule is met.
Section 1.3.1 Schedule of Activities	The following updates were made to the Schedule of Activities: - Obstetric ultrasound Day 1, visit 2 (pre-dose: vaccination 1): footnote 4 deleted - Nasal swab Day 1 visit 2 (pre-dose): footnote 5 deleted.	Correction of an error in the original protocol.
Section 1.1 Synopsis; Section 3 Objectives and Endpoints	Inclusion of analysis of the phenotype of antigen-specific T and B cells, assessed by single cell analysis.	Endpoint erroneously not listed in the original protocol.

Section Number and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis; Section 3 Objectives and Endpoints; Section 8.1.1 Immunogenicity Assessments	Summary of Humoral and Cellular Immunogenicity Assays updated to include updates to the exploratory endpoints for analysis of antibody binding specificities: - To assess in adult participants, the impact of preexisting humoral immunity against coronavirus other than SARS-CoV-2 at baseline, on acquisition of Ad26.COV2.S vaccine immunogenicity: ▪ Analysis of antibodies binding to coronaviruses other than SARS-COV-2 by ELISA or equivalent assay, or to other respiratory viruses (Meso Scale Discovery).	Endpoint erroneously not listed in the original protocol.
Section 2 Introduction	Text updated in line with the latest Investigator Brochure (dated 19 February 2021) and published studies.	Inclusion of latest available information.
Throughout the protocol	Minor grammatical, formatting, or spelling changes were made.	Minor errors were noted

Original Protocol	07 December 2020

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INVESTIGATOR AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study intervention, the conduct of the study, and the obligations of confidentiality.

Coordinating Investigator (where required):

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Signature: _____ Date: _____

(Day Month Year)

Principal (Site) Investigator:

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Telephone Number: _____

Signature: _____ Date: _____

(Day Month Year)

Sponsor's Responsible Medical Officer:

Name (typed or printed): PPD

Institution: Janssen Vaccines & Prevention B.V.

Signature: electronic signature appended at the end of the protocol Date: _____

(Day Month Year)

Note: If the address or telephone number of the investigator changes during the course of the study, written notification will be provided by the investigator to the sponsor, and a protocol amendment will not be required.

Signature

User	Date	Reason
PPD	16-Sep-2022 07:31:52 (GMT)	Document Approval