Janssen Research & Development

Statistical Analysis Plan

A Randomized, Double-blind, Phase 3 Study to Evaluate 6 Dose Levels of Ad26.COV2. S Administered as a Two-Dose Schedule in Healthy Adults

Protocol VAC31518COV3003 Phase 3

VAC31518 (JNJ-78436735 [Ad26.COV2.S])

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

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VERSION HISTORY

Table 1: SAP Version History Summary

SAP version	Approval Date	Change	Rationale
2	15/12/2022	New follow-up 3 has been included which includes follow up from end of post dose 2 period to end of study (section 5.1.2)	Due to the reduction of follow up from 12 to 6 months after second vaccination when some participants already reached 12 months follow-up after second vaccination.
2	15/12/2022	Data from participants from the main study and sub-study will be combined	Due to the unexpected high number of N-serology positive participants during the study to maintain the power for non-inferiority (NI)
2	15/12/2022	Text has been added explaining the change in the sequential order of NI testing (section 2)	The $2.5x10^{10}$ vp dose will be tested before the $7x10^{10}$ vp in the sequential testing due to a smaller sample size in the $7x10^{10}$ vp group (dose not included in the sub-study)
2	15/12/2022	Safety, reactogenicity and immunogenicity data from timepoints that are shared between the main and sub-study will be combined	Same main data collection and timepoints for doses 9x1010 vp, 5x1010 vp, 2.5x1010 vp or 1.25x1010 vp in the main and sub-study
2	15/12/2022	Text has been added explaining two scenarios which will be considered for NI testing (section 4), using only one scenario as primary analysis for NI testing	Due to the unexpected high expected number of N-serology positive during the study, to maintain the power for NI
2	15/12/2022	End of Post-dose 2 period has been updated from 14 days to 28 days after dose 2 vaccination for safety analysis	Accounting for 28 days for safety analysis according to the CTP
2	15/12/2022	Text on new analysis windows has been added (section 5.1.3)	Emerging data from other studies indicating that analysis windows can be extended without impacting immunogenicity outcomes
2	15/12/2022	Text on the definition of a SARS-CoV-2 infection was added (Section 6.1.4)	To clarify the definition of SARS-CoV-2 infections.
2	15/12/2022	Text on descriptive exploratory assessments in the sub study was added.	To expand on the planned assessments as part of the exploratory objectives in the sub-study.
3	04/07/2023	Add imputation rule for Ad26 VNA	The assay was transferred from internal lab to Nexelis and the limits were handled slightly different. The change was made in order to align with other studies and programs.
3	04/07/2023	Edit for wording and formatting	to improve the document readability

1. INTRODUCTION

This statistical analysis plan (SAP) specifies definitions of analysis sets, key derived variables, and the statistical analysis methods for the planned analyses of safety, reactogenicity, and immunogenicity data for the planned primary and final analysis of the study. The primary and final analysis will be performed on unblinded data. The current SAP is based on Clinical Trial Protocol amendment 7 of VAC31518COV3003. Titles, mock-ups, and programming instructions for all statistical outputs (tables, figures and listings) will be provided in a separate document titled Data Presentation Specifications (DPS). This SAP is to be finalized prior to final database lock.

1.1. Objectives and Endpoints for Participants in the Main Study

Objectives	Endpoints
Primary	
To demonstrate non-inferiority (NI) in the following sequential order: • NI after 1-dose of Ad26.COV2.S $9x10^{10}$ vp vs 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 1 dose of Ad26.COV2.S $2.5x10^{10}$ vp vs 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 1 dose of Ad26.COV2.S $7x10^{10}$ vp vs 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 1 dose of Ad26.COV2.S $3.5x10^{10}$ vp vs 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 1-dose of Ad26.COV2.S $1.25x10^{10}$ vp vs 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)	• Binding antibody concentrations to SARS-CoV-2 S protein as measured by ELISA 28 days after first vaccination • NI will be demonstrated in terms of humoral immune response expressed by the GMCs of S-ELISA, 28 days post-dose 1, using a NI margin of 2/3 for the GMC ratio (GMC $9x10^{10}$ vp, $2.5x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, or $1.25x10^{10}$ vp/GMC $5x10^{10}$ vp)
To demonstrate NI in the following sequential order: • NI after 2-doses of Ad26.COV2.S $9x10^{10}$ vp vs 1 dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $9x10^{10}$ vp vs 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)	• Binding antibody concentrations to SARS-CoV-2 S protein as measured by ELISA 14 days after vaccination 2 • NI will be demonstrated in terms of humoral immune response expressed by the GMCs of S-ELISA, 14 days post-dose 2 ($9x10^{10}$ vp, $2.5x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, or $1.25x10^{10}$ vp) and 28 days post-dose 1 or 2 ($5x10^{10}$ vp), using a NI margin of 2/3 for the GMC ratio (GMC [$9x10^{10}$ vp, $2.5x10^{10}$, $7x10^{10}$ vp, $3.5x10^{10}$ vp, or $1.25x10^{10}$ vp)/GMC $5x10^{10}$ vp)

Objectives	Endpoints
• NI after 2-doses of Ad26.COV2.S $2.5x10^{10}$ vp vs 1 dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $2.5x10^{10}$ vp vs 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $7x10^{10}$ vp vs 1 dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $7x10^{10}$ vp vs 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $3.5x10^{10}$ vp vs 1 dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $3.5x10^{10}$ vp vs 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $2.5x10^{10}$ vp vs 1 dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $2.5x10^{10}$ vp vs 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $1.25x10^{10}$ vp vs 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer) • NI after 2-doses of Ad26.COV2.S $1.25x10^{10}$ vp vs 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)	
Secondary	
To assess the humoral immune response and durability to Ad26.COV2.S across all groups, at all immunogenicity blood collection timepoints.	• Serological response to vaccination and binding antibody GMCs to SARS-CoV-2 S protein as measured by ELISA, or equivalent assay

Objectives	Endpoints
To assess the safety and reactogenicity of Ad26.COV2.S administered at several dose levels.	• Solicited local and systemic AEs for 7 days after each vaccination • Unsolicited AEs for 28 days after each vaccination • SAEs throughout the study (from first vaccination until end of the study) • Adverse events of special interest (AESIs [from first vaccination until end of the study]) • MAAEs (until 6 months post-dose 2) • AEs leading to study discontinuation (during the entire study) for all participants following vaccination
Exploratory	
To further explore humoral immune responses to Ad26.COV2.S across all groups 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 (wtVNA and/or psVNA) including neutralization of emerging SARS-CoV-2 • SARS-CoV-2 binding antibodies against emerging SARS-CoV-2 variants as assessed by S-ELISA or equivalent assay • Adenovirus neutralization • Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, immunoglobulin (Ig) subclass and IgG isotype • Analysis of circulating Spike protein • Epitope-specificity characterization of antibodies • Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in the serum or plasma • Passive transfer: Analysis of immune mediators correlating with protection

Objectives	Endpoints
	against experimental SARS-CoV-2 challenge in a suitable animal model
To assess the correlation between the binding antibody (ELISA) titers and neutralizing antibody (VNA) titers to SARS-CoV-2, in a subset of participants at selected timepoints.	• Correlation between ELISA (SELISA; EU/mL) and VNA (wild-type virus [wt]VNA and/or pseudovirion [ps]VNA) titers at selected timepoints
To assess for the occurrence of asymptomatic SARS-CoV-2 infection.	• The number of participants with positive non-S protein ELISA (eg, nucleocapsid [N] protein ELISA).
To examine the immune response in vaccinated individuals with prior or breakthrough infection	• SARS-CoV-2 neutralizing titers in serum measured by a VNA (wild-type virus and/or pseudovirion expressing S protein) • SARS-CoV-2-binding antibodies measured by ELISA: Analysis of antibodies binding to the SARS-CoV-2 S protein
To assess hematology laboratory parameters before and after Ad26.COV2.S administration.	• Including but not limited to: Lupus anticoagulants, anti-β2 glycoprotein, anti-cardiolipin, D-dimers, and anti-PF4

GMC=geometric mean concentration

1.2. Objectives and Endpoints for Participants in the Sub Study

Objectives	Endpoints
Secondary	
To assess the humoral immune response and durability to Ad26.COV2.S across all groups in the sub study, at all blood collection timepoints.	• Serological response to vaccination and binding antibody GMCs to SARS.COV-2 S protein as measured by ELISA, or equivalent assay.
To assess the safety and reactogenicity of Ad26.COV2.S administered at several dose levels	• Solicited local and systemic AEs for 7 days after each vaccination • Unsolicited AEs for 28 days after each vaccination • SAEs throughout the study (from first vaccination until end of the study) • Adverse events of special interest (AESIs [from first vaccination until end of the study]) • MAAEs (until 6 months post-dose 2)

Exploratory	
To further explore humoral immune response to Ad26.CoV2.S across all groups in the sub study 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 (wtVNA and/or psVNA) including neutralization of emerging SARS-CoV-2 variants • SARS-CoV-2 binding antibodies against emerging SARS-CoV-2 variants as assessed by S-ELISA or equivalent assay • Adenovirus neutralization • Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, immunoglobulin (Ig) subclass and IgG isotype • Analysis of circulating Spike protein • Epitope-specificity characterization of antibodies • Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in the serum or plasma
To assess the correlation between the binding antibodies (ELISA) titers and neutralizing antibody (VNA) titers to SARS-COV-2, in a subset of participants at selected timepoints.	Correlation between ELISA (S-ELISA; EU/mL) and VNA (wild-type virus [wt]VNA and/or pseudo virion [ps]VNA) titers at selected timepoints
To assess for the occurrence of asymptomatic SAR-CoV-2 infection.	The number of participants with positive non-S protein ELISA or equivalent assay (e.g., nucleocapsid [N] protein ELISA).
To examine the immune response in vaccinated individuals with prior or breakthrough infection	SARS-CoV-2 neutralizing titers in serum measured by a VNA (wild-type virus and/or pseudo virion expressing s protein) SARS-Cov-2- binding antibodies measured by ELISA: Analysis of antibodies binding to the SARS-CoV-2 S protein
To evaluate the innate, pro-inflammatory and other potentially relevant responses to	Analysis of messenger ribonucleic acid (mRNA) expression levels of vaccine-induced

Ad26.COV2.S vaccination at selected timepoints.	innate responses including inflammatory and coagulation-related mediators. Analysis of cytokines, chemokines, and other proteins- or lipids mediators of the innate immune response.
To assess hematology laboratory parameters before and after Ad26.COV2. S administration.	Include but not limited to: Lupus anti-coagulants, anti-β2 glycoprotein, ant-cardiolipin, D-dimers, and anti-PF4

Ad26= adenovirus 26: GMC= geometric mean concentration

If a correlate or threshold for protection against COVID-19 is established in terms of humoral immunity, then a statistical comparison to that correlate or threshold will substitute non-inferiority testing to immune responses to vaccine at release, as outlined in a revised analytical plan.

1.3. Study Design

Main Study

This is a randomized, double-blind Phase 3 study to evaluate 6 dose levels of Ad26.COV2.S administered as a 2-dose schedule in healthy adults. In this main study, the safety, reactogenicity, and immunogenicity of 1 dose (dose 1 of the 2-dose regimen) and 2-doses of Ad26.COV2.S will be evaluated. The lower dose ($3.5x10^{10}$ vp, $2.5x10^{10}$ vp, and $1.25x10^{10}$ vp) levels mimic vaccine degradation to enable determination of product expiry. In order to increase the end of shelf-life specifications a potential target release titer of $7x10^{10}$ vp will be evaluated. A higher titer of $9x10^{10}$ vp will also be evaluated as is the upper limit of the release range (potential maximum vp at release). The study population will consist of healthy men and women aged between 18 and 55 years (inclusive), who have not previously received a vaccine against COVID-19 and have not had prior exposure to SARS-CoV-2 as assessed by local serology testing. Participants will receive Ad26.COV2.S administered IM.

The study duration from screening until the last follow-up visit will be at least 8 months per participant. The study will consist of a 28-day screening phase with vaccinations on Day 1 and Day 57, and a follow-up of at least 6 months after the last vaccination.

A target of approximately, 1,350 participants (225 participants per active vaccine group) in the main study will be randomized in parallel in a 1:1:1:1:1:1 ratio to 1 of 6 vaccination groups in this study. Participants will receive a 2-dose vaccination regimen at different dose levels (9×10^{10} vp, $2.5x10^{10}$ vp , 7×10^{10} vp, 5×10^{10} vp, $3.5x10^{10}$ vp, and $1.25x10^{10}$ vp).

Table 2: Schematic Overview of Main Study Design and Groups

Group	N	Day 1 Vaccination 1	Day 57 Vaccination 2
1	For NI ~ 225	Ad26.COV2.S $9x10^{10}$ vp	Ad26.COV2.S $9x10^{10}$ vp
2	For NI ~ 225	Ad26.COV2.S $7x10^{10}$ vp	Ad26.COV2.S $7x10^{10}$ vp
3	For NI ~ 225	Ad26.COV2.S $5x10^{10}$ vp	Ad26.COV2.S $5x10^{10}$ vp
4	For NI ~ 225	Ad26.COV2.S $3.5x10^{10}$ vp	Ad26.COV2.S $3.5x10^{10}$ vp
5	For NI ~ 225	Ad26.COV2.S $2.5x10^{10}$ vp	Ad26.COV2.S $2.5x10^{10}$ vp
6	For NI ~ 225	Ad26.COV2.S $1.25x10^{10}$ vp	Ad26.COV2.S $1.25x10^{10}$ vp

Sub Study

Per amendment 5, an additional enrollment of adult participants 18 to 55 years, inclusive, will enroll into the sub study, into group 1, 3, 5 and 6 to further characterize the innate, pro-inflammatory and other relevant (e.g., pro-thrombotic) response to the Ad26.CoV2.S to better understand a possible risk to TTS events. Participants will receive Ad26.CoV2.S administered IM.

A target of approximately 240 participants (approximately 40 seronegative and approximately 20 seropositive participants per dose regimen) will be enrolled in the sub study and will receive a 2-dose vaccination regimen at either $9x10^{10}$ vp, $5x10^{10}$ vp, $2.5x10^{10}$ vp or $1.25x10^{10}$ vp.

Table 3 : Schematic Overview of Study Design and Groups for the Sub Study

Group	N seronegative	N seropositive	Day 1 Vaccination 1	Day 57 Vaccination 2
1	N ~ 40	N ~ 20	Ad26.COV2.S $9x10^{10}$ vp	Ad26.COV2.S $9x10^{10}$ vp
3	N ~ 40	N ~ 20	Ad26.COV2.S $5x10^{10}$ vp	Ad26.COV2.S $5x10^{10}$ vp
5	N ~ 40	N ~ 20	Ad26.COV2.S $2.5x10^{10}$ vp	Ad26.COV2.S $2.5x10^{10}$ vp
6	N ~ 40	N ~ 20	Ad26.COV2.S $1.25x10^{10}$ vp	Ad26.COV2.S $1.25x10^{10}$ vp

2. STATISTICAL HYPOTHESES

The primary objective of this study is to demonstrate the NI, in terms of humoral immune response, of 1-dose and 2-doses of $9x10^{10}$ vp, $2.5x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, and $1.25x10^{10}$ vp Ad26.COV2.S versus 1-dose and 2-doses of $5x10^{10}$ vp Ad26.COV2.S (release titer).

Formal NI testing will be applied to demonstrate NI of 1-dose and 2-doses of $9x10^{10}$ vp, $2.5x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp and $1.25x10^{10}$ vp Ad26.COV2.S versus 1- dose and 2-doses of $5x10^{10}$ vp Ad26.COV2.S (release titer), using a NI margin of 2/3 for the GMC ratios.

The following 2 sets of hypotheses (1 dose and 2 doses) will independently be tested sequentially:

Post-dose 1

1a) NI after 1-dose of Ad26.COV2.S $9x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

1b) NI after 1-dose of Ad26.COV2.S $2.5x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

1c) NI after 1-dose of Ad26.COV2.S $7x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

1d) NI after 1-dose of Ad26.COV2.S $3.5x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

1e) NI after 1-dose of Ad26.COV2.S $1.25x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

In this set, NI will be demonstrated in terms of humoral immune response expressed by the GMCs of S-ELISA, 28 days post-dose 1, using a NI margin of 2/3 for the GMC ratio (GMC [$9x10^{10}$ vp, $2.5x10^{10}$ vp , $7x10^{10}$ vp, $3.5x10^{10}$ vp, or $1.25x10^{10}$ vp]/GMC $5x10^{10}$ vp).

Post-dose 2

2a1) NI after 2-doses of Ad26.COV2.S $9x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2a2) NI after 2-doses of Ad26.COV2.S $9x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2b1) NI after 2-doses of Ad26.COV2.S $2.5x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2b2) NI after 2-doses of Ad26.COV2.S $2.5x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2c1) NI after 2-doses of Ad26.COV2.S $7x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2c2) NI after 2-doses of Ad26.COV2.S $7x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2d1) NI after 2-doses of Ad26.COV2.S $3.5x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

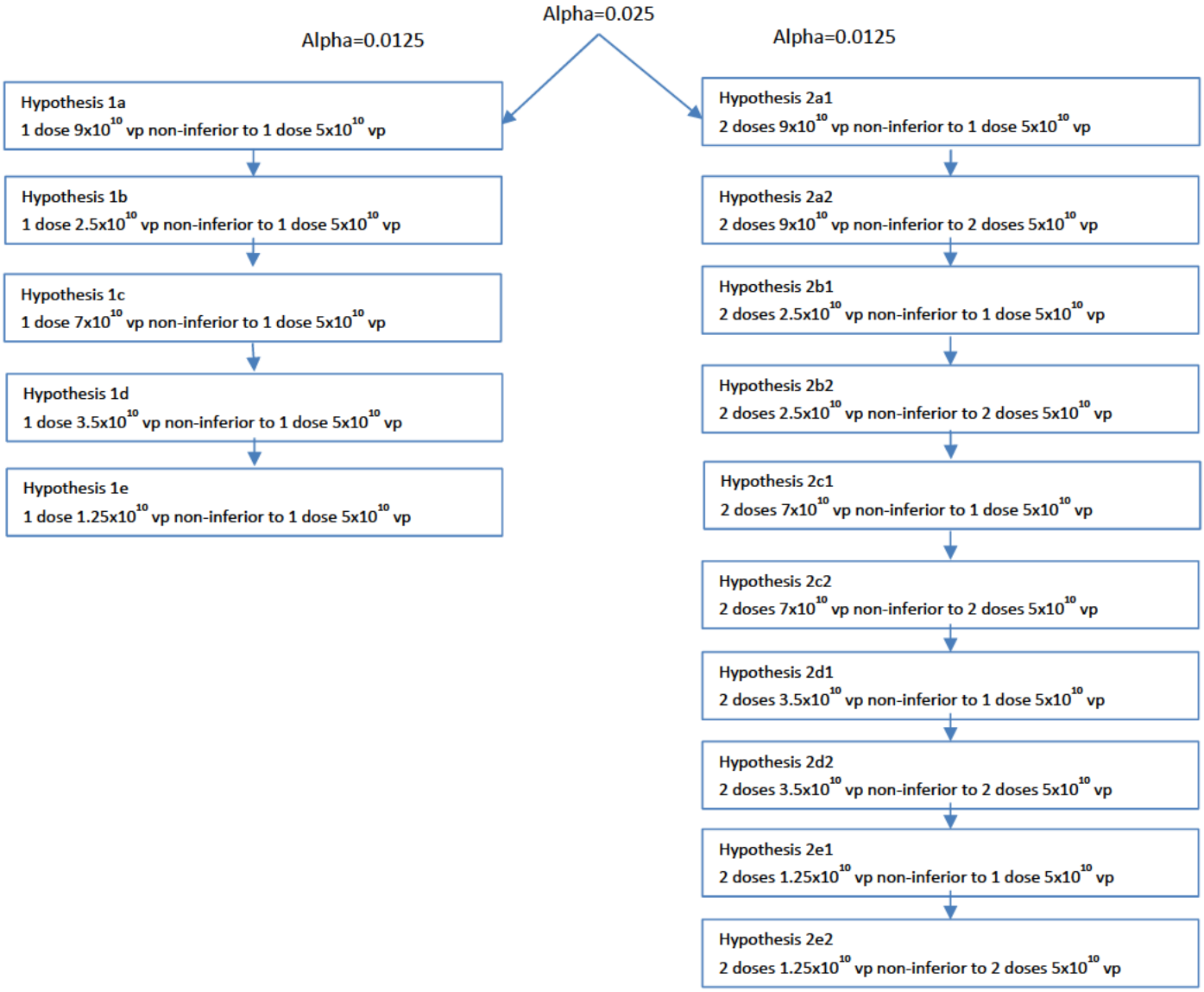
2d2) NI after 2-doses of Ad26.COV2.S $3.5x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2e1) NI after 2-doses of Ad26.COV2.S $1.25x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2e2) NI after 2-doses of Ad26.COV2.S $1.25x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

In this set, NI will be demonstrated in terms of humoral immune response expressed by the GMCs of S-ELISA, 14 days post-dose 2 ($9x10^{10}$ vp, $2.5x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, or $1.25x10^{10}$ vp) and 28 days post-dose 1 or 14 days post-dose 2 ($5x10^{10}$ vp), using a NI margin of 2/3 for the GMC ratio (GMC [$9x10^{10}$ vp, $2.5x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, or $1.25x10^{10}$ vp]/GMC $5x10^{10}$ vp below depicts a tree-based schema for testing the 2 sets of non-inferiority hypotheses (1 dose and 2 doses) controlling the family-wise error rate at alpha = 0.025 (1-sided)

Figure 1: Decision Tree-Based Hypothesis Testing



For all comparisons, an NI margin of 2/3 for the GMC ratio will be used. Multiplicity adjustments and more details on the analyses are provided in Section 5.

3. SAMPLE SIZE DETERMINATION

The number of participants chosen is to provide sufficient power for the non-inferiority comparisons.

The following assumptions were made in the sample size determinations:

Log transformed (log_{10} scale) immune response data are normally distributed

A common standard deviation (SD): SD=0.5 (log_{10} scale) of the immune response log transformed data

Non-inferiority margin = $log_{10}(2/3)$ = -0.176

Alpha = 0.0125 (1-sided) for 1- and 2-dose NI comparisons

- Alpha = 0.025 (one-sided). Using a Bonferroni correction, Type I error will be split equally over the 1-dose and 2-dose comparisons: 0.0125 (1-sided) for 1- and 2-dose NI comparisons

Power = 90%

Based on the above assumptions, and approximately a 10% dropout, a sample size of 225 participants per active vaccine group (see Table 2) is needed to detect non-inferiority of testing 1-dose and 2-doses of $9x10^{10}$ vp, $2.5x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, and $1.25x10^{10}$ vp Ad26.COV2.S as the test groups versus 1-dose and 2-doses of $5x10^{10}$ vp Ad26.COV2.S (release titer) as the reference groups, with 90% power (main study sample size assessment).

The originally intended NI population was comprised of participants from the main study who were SARS-CoV-2 seronegative at baseline and non-previously vaccinated. In addition, participants becoming infected with SARS-CoV-2 during the study are excluded from this population from the time of infection.

Participants were screened using a local SARS-CoV-2 serology test to ensure only participants who were seronegative at baseline were enrolled in the main study. However, a preliminary blinded evaluation of central N-serology results in main study participants at Day 29 and Day 71 showed that overall, 27% and 38% of participants, respectively, were N-serology positive at these time points. The higher-than-expected N-serology positive status at Day 29 and Day 71 indicated that participants became infected during the study and/or local serology tests were not sufficiently sensitive, affecting the power of the study.

For the sub-study (initiated to further characterize the innate, pro-inflammatory and other relevant (e.g., prothrombotic) responses to Ad26.COV2.S to better understand a possible risk to TTS events), a target of approximately 240 participants will be enrolled and will receive a 2-dose vaccination regimen at either 9×10^{10} vp, $2.5x10^{10}$ vp, 5×10^{10} vp, or $1.25x10^{10}$ vp. As participants in the sub-study share common blood sampling timepoints with the main study, data from both cohorts can be merged for analyses. Due to the higher-than-expected N serology positive results in the main study affecting the power of the study, the non-inferiority assessment will combine participants from the main study and sub-study.

Table 4 below shows how, when combining main and sub study participants (randomized as SARS-CoV-2 seronegative at baseline based on local serology by finger prick testing), the power is affected due to a hypothetical 10%, 20%, 30%, 40%, 50% and 60% loss of evaluable participants (lost due to various reasons, e.g. lost to follow-up, participants with major protocol deviations with impact on immunogenicity, participants who become infected with SARS-CoV-2, participants who received their vaccination outside the allowed window, etc.).

Table 4: Impact of loss of evaluable subjects on NI assessment power

			% loss of evaluable participants since enrollment					
Dose level	**Sample size at enrollment per arm (main study + sub study)**	**Group**	**10%**	**20%**	**30%**	**40%**	**50%**	**60%**
9x1010 vp	265(225+40)	1	94%	91%	87%	81%	73%	62%
2.5x1010 vp	265(225+40)	5	94%	91%	87%	81%	73%	62%
7x1010 vp	225 (225+0)	2	90%	86	81%	74%	65%	54%
5x1010 vp	265(225+40)	3	94%	91%	87%	81%	73%	62%
3.5x1010 vp	225 (225+0)	4	90%	86	81%	74%	65%	54%
1.25x1010 vp	265(225+40)	6	94%	91%	87%	81%	73%	62%

4. POPULATIONS (ANALYSIS SETS) FOR ANALYSIS

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

Analysis Sets	Description
All screened participants (ALL)	The "all screened participants" set includes all participants that were screened, regardless of whether they were enrolled and/or randomized.
All randomized participants (ALL RANDOMIZED)	The "all randomized participants" set includes all participants that were randomized to one of the treatment groups.
Full Analyses Set (FAS)	The Full Analysis Set will include all participants with at least one vaccine administration documented
Per Protocol Immunogenicity Set (PPI)	The Per Protocol Immunogenicity population will include all randomized and vaccinated participants for whom immunogenicity data are available excluding participants with major protocol deviations expected to impact the immunogenicity outcomes (see section 4.1). In addition, samples obtained after missed vaccinations or samples obtained from participants after SARS-CoV-2 infection occurring after screening (if applicable) will be excluded from the analysis set (see below section 4.1).
NI Analysis Set	The NI analysis set will include all PPI participants who are SARS-CoV-2 seronegative at study entry[a].

[a] SARS-CoV-2 seronegative as assessed by central testing (based on S- and/or N- serology at baseline)

NB. As detailed in Section 4, formal non-inferiority hypothesis testing will be performed on either the NI set or on the PPI set under different scenarios (Scenario 1 or Scenario 2, respectively).

Further details on the selection of PPI and NI set are described below.

The safety and reactogenicity analyses will be censored at the date of unblinding or the receipt of an unscheduled vaccine, whichever event occurs first. AEs reported after unblinding or receipt of an unscheduled vaccine, whichever comes first, will be listed.

The immunogenicity analyses will be censored at the date of the receipt of an unscheduled vaccine. Immunogenicity samples taken after the receipt of an unscheduled vaccine will be included in the listings and flagged.

If a participant is vaccinated out of window (see section 1.4.1 of the protocol) due to a study pause (see section 6.9 of the protocol), this will not by default be a reason for excluding this participant from the PPI. 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.

4.1. Selection of PPI dataset

- Samples obtained from participants after SARS-CoV-2 infection occurring after screening (if applicable) will be excluded from the analysis set.
  - This includes COVID-19 cases with a positive RT-PCR or molecular test, but also participants who develop a post-baseline positive result in non-S-based serology (e.g. N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay) if the test was negative at baseline. Samples at the time of positive RT-PCR or molecular test, or non-S-based serology and thereafter will be excluded from PPI.
  - Participants with missing or having undetermined SARS-CoV-2 serostatus at baseline will be excluded from PPI.
- Major Protocol Deviations (MPDs) with impact on immunogenicity will be considered for the PPI population. MPDs are defined in the MPD Criteria document. Different rules are applicable for selected MPDs with impact on immunogenicity:
  - For MPDs with impact on immunogenicity which concern inclusion or exclusion criteria (category: entered but did not satisfy criteria), the whole subject will be excluded from PPI.
  - For MPDs with impact on immunogenicity which can affect immunogenicity outcomes from the time of PD and thereafter, such as missed vaccinations, out of window vaccinations (Day 57 visit), use of disallowed concomitant medications or off-study COVID-19 vaccines (categories: received a disallowed concomitant treatment, received wrong treatment or incorrect dose, developed withdrawal criteria but no withdrawn, in addition to ‘missed vaccination’ under the category ‘Other’, and ‘received vaccination outside of visit window’ under the category ‘Dosage & Administration’), any samples obtained at the time of PD and thereafter will be excluded from PPI. Samples taken from the subject prior to such PDs can be included in PPI.
  - For MPDs with impact on immunogenicity which can affect immunogenicity outcomes only at the time of PD, such as out of window visits or blood collections (category: Other except for missed vaccination or out of window vaccinations which is included in the rule above), samples obtained at the time of PD will be excluded from PPI only. Samples taken from the subject prior to or after such PDs can be included in PPI.
  - The exact PD rules may be amended, or other PD types may be added, this will be further described in the DPS. For PDs relating to out of window visits or sampling, the windows will be defined as indicated in Section 5.1.3.

4.2. Selection of NI dataset

Decision for selection of population for non-inferiority testing

Participants were screened using a local SARS-CoV-2 serology test to ensure that only participants who were seronegative at baseline were enrolled in the main study (or randomized as seropositive

or seronegative at baseline in the sub study). Prior to Primary Analysis, a preliminary blinded evaluation of central N-serology results in main study participants at Day 29 and Day 71 showed that overall, 27% and 38% of participants, respectively, were N-serology positive at these time points. The higher-than-expected N-serology positive status at Day 29 and Day 71 indicated that participants became infected during the study and/or local serology tests were not sufficiently sensitive, affecting the power of the study.

While data following SARS-CoV-2 infections during the study will be excluded from non-inferiority assessments, data from participants who are seropositive at baseline as assessed by central testing could be included in the non-inferiority assessment if serostatus at baseline is considered as an independent variable (scenario 2).

Based on these considerations, only one of two scenarios will be considered for primary analysis. If there is an overall loss of > 40% evaluable data points in the NI set at Day 29 (defined as number of evaluable participants in the NI set at D29/total number of subjects in Table 4), the population in scenario 2 will be used for primary analysis.

Scenario 1 (base case scenario): non-inferiority will be performed on the NI set including participants from the main study and sub-study.

Scenario 2: Non-inferiority analysis will be performed on the PPI set including participants from the main study and sub-study. In this scenario a model accounting for SARS-CoV-2 serostatus at baseline will be used.

5. STATISTICAL ANALYSES

5.1. General Considerations

5.1.1. Study Phases

A baseline (or reference) value will be defined as the value of the last available assessment prior to the first vaccination on Day 1, unless specified otherwise. The safety analysis will present all results by phase (see Section 5.2).

Immunogenicity results will be presented per scheduled time point as appropriate using visit windows (see section 1.4 in the protocol). Listings will be presented per phase and time point.

Study day or relative day is defined as follows:

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

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

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

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

relday = visit date – reference date for visits before the reference,

where the reference date equals the date of vaccination 1 or 2.

The planned primary and final analysis will be performed on unblinded data. During primary analysis data will be unblinded to the sponsor.

5.1.2. Phase Definitions

The phases in the study will be constructed, as follows in Table 5

Table 5: Phase Definitions

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

					c) One minute prior to 6 months after second vaccination
Follow-up 3	5			One minute after post-dose 2 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) One minute prior to the date of unblinding visit or database cut-off date, whichever occurs first c) 23:59 at the date of last visit

Note: the end of regimen phase should be the end of the last post dose period
FU3: Full follow-up from end of post dose 2 to end of study or last contact
Post- dose 1 and post dose 2 Combined includes events occurring in the post-dose 1 and/or post dose 2 phases (not including FU 1 or FU2 or FU3)
For final analysis the following criteria for Follow-Up 3 will be used 23;59 at date of last contact (for early discontinuation or date of last visit)

5.1.3. Visit Windows

Per-protocol visit windows (refer to CTP section 1.4) guide study procedures. Analysis visit windows are defined considering the emerging data from other studies relating to sampling time points and potential impact on immunogenicity. As such, analysis windows may be redefined prior to unblinding. No time windows apply for Early Exit visit sampling.

The following visit window rules will be considered for the analysis of immunogenicity results:

For vaccination:

1. If a participant missed a scheduled vaccination, all immunogenicity samples taken after the missed vaccination will be excluded from the PPI. Samples taken before will still be included in the PPI.
2. If the scheduled vaccination at vaccination visit Day 57 is ≥ -9/+16 days from planned vaccination date (outside Day 49- Day 72), all immunogenicity samples taken after will be excluded from the PPI. I.e., if the vaccine is administered at least 9 days before or at least 16 days after the planned vaccination date, all immunogenicity samples taken on or after will be excluded from the PPI set.

For immunogenicity sampling (excluding D2, D4, D8, D58, D60 and D64 for sub-study participants):

1. If immunogenicity sample is taken out of window when planned within three months (≤ 3 months) post dose 1 vaccination (i.e. up to and including Day 71 visit), the sample will be excluded from PPI as follows:
    a. For sampling at D29 if taken ≥-/+9 days from the planned Visit date.
    b. For sampling at D57 if taken ≥-9/+16 days from the planned Visit date.
    c. For sampling at D71 if taken ≥-4/+16 days from the planned Visit date.

For example, samples outside the following windows will be excluded:

a) Post Vac 1 + 28-day visit (D29): samples taken outside 21 days and up to 37 days post vaccination 1 will be excluded.

b) Vac 2 visit (D57): samples taken outside 49 days and up to 72 days post vaccination 1 will be excluded.
c) Post Vac 2 + 14 day visit (D71): samples taken outside 11 days and up to 29 days post vaccination 2 will be excluded.
d) Other samples from these participants can still be included in PPI.

2. If immunogenicity sample is taken out of window when planned 24 weeks post last vaccination and thereafter, this sample will be excluded from PPI if taken ≥-/+ 30 days from Visit timing date. Other samples from this participant can still be included in PPI.

For early immunogenicity sampling in the sub-study (includes only D2, D4, D8, D58, D60 and D64 for sub study participants): If immunogenicity sample is taken outside of the protocol-defined windows as defined in CTP 1.4.2 schedule of activities, these samples will be excluded from the PPI.

5.1.4. Pooling Algorithm for Analysis Centers

Data will be pooled across the different centers.

5.1.5. Definition of Subgroups

The following subgroups will be investigated for immunogenicity: Sex, Age group, SARS-CoV-2 Serostatus at baseline and race. For selected safety and immunogenicity assessments, subgroup analysis will include age group, serostatus and BMI. Sub-group analysis for serostatus at baseline will only be done if at least ~10% of participants are seropositive at baseline. Final analysis for immunogenicity may include subgroups analyses for SARS-CoV-2 infection during the study and for off-study COVID-19 vaccination.

Subgroup	Definition
BMI	• underweight <18.5 kg/m2 • normal 18.5-<25 kg/m2 • overweight 25-<30 kg/m2 • obese >=30 kg/m2
Age Group 1	• 18-40 • 41-55
Age Group 2	• 18-25 • 26-40 • 41-55
Ethnicity	• Hispanic or Latino, • Not Hispanic or Latino, • Not Reported • Unknown
Sex	• Male • Female • Intersex • Unknown
Race[a]	• American Indian or Alaska Native • Asian • Black or African American

Subgroup	Definition
	• Native Hawaiian or other Pacific Islander • White • Not reported • Unknown
SARS-CoV-2 Serostatus[b] at baseline	• Positive • Negative • Missing
SARS-CoV-2 infection[c] during study	• Yes • No
Off-study COVID-19 vaccination during the study	• Yes • No

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

[b] Serostatus at baseline will be based on central testing, namely S and/or non-Spike-based serology (e.g. N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay), as available, and is defined as:

- Positive for participants who are positive at baseline by S-ELISA, and/or non-S-based serology (e.g. N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay), as available.
- Negative for participants who are negative at baseline by S-ELISA, and non-S-based serology (e.g. N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay), if available.
- Missing if data on all of the above assays are missing, or if one of the assays is missing and the other is negative.

[c] SARS-CoV-2 infection is defined as a positive SARS-CoV-2 PCR test or conversion from a negative at baseline to positive post-baseline result with a non-Spike protein-based serology test (e.g. N serology).

Additional subgroup analyses might be performed for safety or immunogenicity if it is deemed useful to the study objectives.

5.2. Participant Disposition

Participant information will be shown for all analysis sets.

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

- participants screened
- participants randomized
- participants in the FAS
- participants vaccinated and not randomized
- participants randomized and not vaccinated
- participants in the PPI
- participants who discontinued study
- participants who discontinued vaccination
    - reasons for termination
- participants who were unblinded during the study period

Also, the number of participants, median follow-up duration and percentage per phase will be tabulated.

5.2.1. Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized.

Table 6 presents a list of the demographic and baseline variables that will be summarized by vaccine regimen and overall for the FAS. This will be done for both the main study and sub study separately.

Table 6: Demographic Variables

Continuous Variables:	**Summary Type**
Age (years)	Descriptive statistics: N, mean, standard deviation [SD], median and range [minimum, maximum].
Weight (kg)	
Height (cm)	
Body Mass Index (BMI) (kg/m^2)	
Categorical Variables	
Sex (male, female, Intersex, Unknown)	Frequency distribution with the number and percentage of participants in each category.
Age group (18-<40 years or 40-<=55 years)	
Race[a] (American Indian or Alaska Native, Asian, Black, or African American, Native Hawaiian or other Pacific Islander, White, Multiple, Not Reported)	
Ethnicity (Hispanic or Latino, not Hispanic or Latino, Not Reported, Unknown)	
Country	
BMI (underweight: <18.5 kg/m^2, normal: 18.5-<25 kg/m^2, overweight: 25-<30 kg/m^2, obese: >=30 kg/m^2)	
SARS-CoV-2 Serostatus[b](Positive / Negative/Missing)	

[a] If multiple race categories are indicated, the Race is recorded as 'Multiple'
[b] Serostatus at baseline will be based on central testing, namely S and/or non-S-based serology (e.g. N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay), as available, and is defined as:

- Positive for participants who are positive at baseline by S-ELISA, and/or non-S-based serology (e.g. N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay), as available.
- Negative for participants who are negative at baseline by S-ELISA, and non-S-based serology (e.g. N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay), as available.
- Missing if data on all of the above assays are missing, or if one of the assays is missing and the other is negative.

5.2.2. Extent of Exposure

The number and percentage of participants who receive study vaccination will be summarized by vaccine group and by visit (Day 1 and Day 57). This will be done for both the main study and sub study separately.

Total Exposure:

Total exposure expressed by the total number of vaccinations received will also be summarized for the counts 1 or 2 vaccine doses by vaccine group.

5.2.3. Protocol Deviations

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

5.2.4. Prior and Concomitant Medications

The analysis of concomitant therapies will be done using the WHO drug coded terms. Based on their start and stop date, concomitant therapies will be reported in each applicable phase.

For all participants, concomitant therapies associated with an SAE will be collected and recorded in the eCRF from the moment of first vaccination through the end of the study. Concomitant therapies associated with MAAEs will be collected and recorded in the eCRF from the moment of first vaccination until 6 months after 2nd vaccination. Concomitant therapies associated with MAAEs leading to study discontinuation will be recorded in the eCRF during the entire study. The proportion of participants with concomitant medication associated with these SAEs and MAAEs will be tabulated and listed.

For all participants, concomitant therapies such as, but not limited to, analgesic/antipyretic medications and non-steroidal anti-inflammatory drugs, corticosteroids, antihistamines, and vaccinations will be recorded from the moment of first vaccination until 28 days after administration of study vaccine, and thereafter, pre-dose on the day of any subsequent vaccination and for 28 days after that vaccination. The proportion of participants with concomitant medication will be tabulated by period.

For all participants, other concomitant therapies will also be recorded if administered in conjunction with a confirmed COVID-19 case or with new or worsening AEs reported per protocol requirements outlined in section 4.2. The proportion of participants with concomitant medication associated with these cases will be tabulated and listed.

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

If a concomitant therapy record misses components of its start and/or stop dates (time, day and/or month and/or year):

- In case of partial start or stop dates, the concomitant therapy records will be allocated to periods using the available partial information, without imputations. If, for example, only month and year are available, these will be compared to the month and the year of the periods, and the concomitant therapy record will be allocated to the period(s) where these date parts match. This rule may lead to assignment to multiple periods.
- In case of a completely missing start date, the concomitant therapy will be considered as having started before the trial.
- In case of a completely missing end date, the concomitant therapy will be considered as ongoing at the end of the trial.

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

5.3. SAFETY

No formal statistical testing of safety data is planned. Safety data will be analyzed descriptively by vaccine group. In addition, for selected tables, tabulations pooled by vaccine dose will also be provided. All safety analyses will be done on the FAS. This will be done for both the main study and sub study separately.

5.3.1. Safety Endpoints

The Safety endpoints are:

Main study:

- Solicited local and systemic AEs for 7 days after each vaccination
- Unsolicited AEs for 28 days after each vaccination
- SAEs throughout the study from first vaccination until end of the study
- Adverse events of special interest (AESIs) from first vaccination until end of the study
- MAAEs until 6 months post-dose 2
- AEs leading to study discontinuation (during the entire study) for all participants following vaccination

Sub study

- Solicited local and systemic AEs for 7 days after each vaccination
- Unsolicited AEs for 28 days after each vaccination
- SAEs throughout the study from first vaccination until end of the study
- Adverse events of special interest (AESIs) from first vaccination until end of the study
- MAAEs until 6 months post-dose 2

The following section provides AE definitions associated with safety and reactogenicity endpoints.

5.3.2. Adverse Events

5.3.2.1. Definitions

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

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

until 6 months after each vaccination, except for MAAEs leading to study discontinuation which are to be reported during the entire study.

Asymptomatic or undetected SARS-CoV-2 infection is defined as a participant does not fulfil the criteria of suspected COVID-19 based on sign and symptoms which would classify them as mild, moderate, or severe by the definition, and has a SARS-CoV-2 positive RT-PCR or molecular test result from any available respiratory tract sample(e.g., nasal swab sample, sputum sample, throat swab sample, saliva sample) or other sample or develops a positive serology(non-S protein) test then, the participant will be considered to have experienced asymptomatic or undetected COVID-19.

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

For AESI, the following subcategories are defined:

1) Suspected AESI as reported by investigator
2) Suspected AESI identified through SMQ search

   Those will include all reported AEs that are identified by the selection rule:

   - SMQ (Standardized MedDRA Queries) = “EMBOLIC AND THROMBOTIC EVENTS (SMQ)”

     or

   - (SUB_SMQ1 = “HAEMATOPOIETIC THROMBOCYTOPENIA (SMQ)” and Scope in (“BROAD”, “NARROW”)) or HLT (higher level term) = “Thrombocytopenias”

3) Suspected AESI qualified for assessment

   Suspected AESIs (reported by investigator / SMQ search) that have risk levels assessed by one of the following three criteria are considered “qualified for assessment”:

   o Brighton Collaboration Level (Level 1-5)
   o CDC Tier (non-tier 1/2, tier 1, tier 2)
   o PRAC criteria (confirmed, possible, probable, unlikely, criteria not met)

The suspected AESI will be reported from the moment of vaccination until the end of the study/early withdrawal. An AESI adjudication Committee with appropriate expertise is established to evaluate each suspected AESI and determine cases of TTS.

5.3.2.2. Severity Criteria

All AEs and laboratory data will be coded for severity using a modified version of the FDA grading table, based on the version of September 2007 (US DHHS FDA CBER 2007), included in Appendix 2, Toxicity Grading Scale.

For AEs not identified in the grading table, the Severity Criteria guidelines in CTP (section 10.4.3) will be applied.

The severity of solicited signs and symptoms will be graded in the reactogenicity diary by the participant based on the severity assessment provided in the reactogenicity diary and then verified by the investigator using the toxicity grading scale in Appendix 2. (Note: severity of the measured events will be derived from the diameter [for erythema and swelling] and the temperature measurements [for fever]).

5.3.3. Analysis of Adverse Events

The number and percentage of participants with at least one AE (unsolicited/solicited) will be tabulated. Unsolicited AEs will be summarized by System Organ Class and Preferred Term. Solicited AEs will be summarized by class (administration site, systemic) and preferred term.

For solicited AEs, the following tables will be provided: summary, by worst severity grade, at least grade 3, related (systemic only), time to onset (in days) and duration (in days) for most frequent events (>1%) and body temperature. Note: Duration is defined as number of days from the start of the event until resolution of the event. The time to first onset is defined as (date of first onset – reference date + 1). The reference date is the start date of the first vaccination period.

For unsolicited AEs, the following tables will be provided: summary table (including SAE, MAAEs, MAAEs leading to study discontinuation, fatal outcome, and discontinuation), all events, most frequent (>1%), at least grade 3, related and SAE. The proportion of AEs resulting in a medically attended visit (other than routine health maintenance visits) will also be tabulated.

For AESI, the following summary tables will be provided on suspected and qualified for assessment of AEs of Special Interest (AESI) by vaccine group:

1. Number of Suspected AE of Special Interest (AESI) (as Reported by Investigator) by SMQ and Preferred Term.

   Note: Suspected AESI as reported by investigator and presented by: Embolic and thrombotic events (SMQ); Haematopoietic thrombocytopenias (SMQ) (broad) or HLT Thrombocytopenias; Other SMQ.

2. Number of Suspected AE of Special Interest (AESI) (Identified through SMQ search) by SMQ and Preferred Term.

   Note: Suspected AESI Identified through SMQ search: Embolic and thrombotic events (SMQ), Haematopoietic thrombocytopenias (SMQ) (broad) or HLT Thrombocytopenias and presented by: Embolic and thrombotic events (SMQ) + sub-SMQ1 + PT; Haematopoietic thrombocytopenias (SMQ) (broad) or HLT Thrombocytopenias + PT;

3. Number of AESI Qualified for assessment by SMQ and Preferred Term.
   Note: AESI Qualified for assessment: [Embolic and thrombotic events (SMQ)] and [Haematopoietic thrombocytopenias (SMQ) (broad) or HLT Thrombocytopenias or platelet count below normal ranges per local or central laboratory report or platelet count < 150 × 10E9/L]

Details on AESIs of thrombosis with thrombocytopenia is specified in section 8.3.6.1 of the CTP. The list of thrombotic events to be reported to the sponsor as suspected AESIs are provided Appendix 9 of the CTP. AEs meeting the suspected AESI criterion will be flagged as such in the

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

5.3.3.1. Phase Allocation of Adverse Events

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

Step 1: Allocation of events to the periods:

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

- In case of partial start or stop dates (i.e. time and/or day and/or month and/or year missing), the events are allocated to the periods using the available partial information on start and end date; no imputation will be done. If, for instance, the AE start date only month and year are available, these data are compared to the month and year information of the periods. This rule may lead to multiplication of the event as a consequence of its assignment to multiple periods.

- In case of a completely missing end date, the date is imputed by the cut-off date of the analysis for participants still ongoing in the study, and by the end date of the last period for participants who discontinued or completed the trial. In case of a completely missing start date, the event is allocated to the first active treatment phase (post dose 1 period), except if the end date of the AE falls before the start of the first active treatment phase (post dose 1 period).

Step 2: Combination of events:

Overlapping/consecutive events are defined as events of the same participant with the same preferred term which have at least 1 day overlap or for which the start date of an event is 1 day after the end date of the preceding event. Overlapping/consecutive events may be combined into one AE or not, according to the following rules:

1. If overlapping/consecutive events start in one of the following periods - Screening or post dose extension (i.e. non-active periods) - followed by an AE in - post-dose period (active period) - they are allocated to their respective periods and are considered as separate events.
2. In case overlapping/consecutive events start within a single period, they are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM data base but are assigned the same onset, period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.
3. In case overlapping/consecutive events start in both an active period followed by a non-active period, they are allocated to the active period only and are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM data base but are assigned the same onset, treatment period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.

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

Remarks:

1. Events can only be combined into one and the same AE if their start and stop dates are known.
2. In case the completely missing end date is imputed (for period allocation), this date is also considered as a complete date.
3. Time is not considered when determining overlap of events.

5.3.3.2. Missing Data

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

5.3.4. Laboratory, Vital Signs and Physical Examination

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

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

Hematology laboratory parameters before and after Ad26.COV2.S administration.

Including but not limited to: Lupus anticoagulants, anti-β2 glycoprotein, anti-cardiolipin, D-dimers, platelet count and anti-PF4

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

Vital signs including temperature, pulse/heart rate, respiratory rate, and blood pressure (systolic and diastolic) will be summarized over time, using descriptive statistics. Abnormalities emerging after vaccination will be tabulated by worst abnormality grade using the FDA table in Appendix 2.

An abnormality (toxicity grade or abnormality based on normal ranges) will be considered as emerging in a particular period if it is worse than the baseline value. If the baseline is missing, the abnormality is always considered emerging. A shift from ‘abnormally low’ at baseline to ‘abnormally high’ post baseline (or vice versa) is also emerging. In case a laboratory test result is censored (no numeric value is available, but only a verbatim term) then a numeric value will be

imputed by a value exceeding the cut-off value with one unit. (<x: subtract 1 unit from x, >x: add 1 unit to x; <3.45 is imputed with 3.44).

In case no toxicity grades are defined for a test, the abnormalities (above/below normal range) will be used. In determining toxicity grades, the following rules are applied:

-worst grades/abnormalities are determined over the whole observational period for each trial period separately, including all post-baseline measurements of that period.

- The abnormalities 'abnormally low' and 'abnormally high' are considered equally important, i.e. if a participant has as well an abnormally low as an abnormally high value post-baseline, both abnormalities are shown in the tables. (This means that the sum of the percentages can be more than 100%)

- Note: as the grading scale for some parameters in the grading table has some gaps (zones where no toxicity grade definition exists), laboratory results falling in these zones will be allocated to the adjacent worst-case grade.

- If a lab value falls within the grading as specified in the grading table but also within the local lab normal limits, the value is considered as normal.

For the grades, no distinction will be made between test results of samples obtained under fasting and under non-fasting conditions: in case limits under fasting and non-fasting conditions differ, the limits of the conditions (fasting/non-fasting) of scheduled visits as planned in the CTP will always be used, also for samples obtained under a different condition (e.g. samples of withdrawal visits).

A listing of participants with fever according to the FDA grading table will also be provided. In addition, temperature measurements (whether obtained from the diary or from on-site assessments) will be allocated to predefined temperature intervals (from 37.5° C until 40°C, in steps of half degree increments, e.g. <37.5, 37.5-<38, 38-<38.5, … >40), and tabulated.

5.4. Immunogenicity

5.4.1. General Considerations

Immunogenicity results will be presented by vaccine group and per scheduled time point as appropriate using visit windows (see Section 5.1.3).

For the PPI analysis samples taken outside of the allowed window as described in section 5.1.3 will be excluded from the table and graphs (but will be included in the listing and clearly marked as results not included in the PPI analyses). For the FAS analysis, samples taken outside of the allowed windows will be included in the tables and graphs.

Listings will be presented by vaccine group and per phase and analysis time point.

Key immunogenicity subgroup analyses will also be performed by BMI, ethnicity, race, sex, participants who were infected with SARS-CoV-2 during the study or those that received an alternative vaccine. Categorical variables will be summarized with a frequency table showing counts and percentages. This will be done for both the main study and sub study separately. Continuous variables will be summarized using the following statistics, as appropriate:

number of observations, geometric mean, arithmetic mean (mean), 95% confidence interval (CI) for the mean, standard deviation (SD), standard error (SE), median, quartiles (Q1 and Q3), minimum and maximum. This will be done for both main study and sub study separately.
Data listings, subject profiles and/or subject narratives may be provided as appropriate. This will be done for both main study and sub study separately.

Binary variables will be summarized using the following statistics: number of observations, percentages and Exact Clopper-Pearson 95% confidence interval (CI). This will be done for both main study and sub study separately.

5.5. Primary Humoral Immune Response Endpoints

5.5.1. Definition of Endpoint(s)

S-ELISA will be used as a primary immunogenicity endpoint, for formal NI testing, to demonstrate non-inferiority for each of the hypothesis tests between the groups as stated in Section 2. This analysis will be based on:

Binding antibody concentrations to SARS-CoV-2 S protein as measured by ELISA 28 days after vaccination 1.

Binding antibody concentrations to SARS-CoV-2 S protein as measured by ELISA 14 days after vaccination 2.

GMCs derivations will be based on the Log transformed (log_{10} scale) immune response (ELISA concentrations) 28 days post-dose 1- and 14-day post-dose 2 data.

NI of the statistical hypotheses (Section 1.3) will be demonstrated in terms of humoral immune response expressed by the GMCs of ELISA, using an NI margin of 2/3.

5.5.2. Estimand

Not applicable

5.5.3. Analysis Methods

Formal non-inferiority hypothesis testing will be performed on either the NI set (Scenario 1) or on the PPI set (Scenario 2) as described in Section 4.

Descriptive non-inferiority and additional immunogenicity assessments may be performed on other populations and subgroups (e.g., PPI, or on participants in the PPI who are SARS-CoV-2 seropositive at baseline, etc.).

Immunogenicity analyses may also be done on the FAS (participants who became infected during the study will be analyzed as a subgroup and will be shown in the graphs using different colors

and symbols). Samples taken outside of the allowed windows as described in section 5.1.3 (included in the FAS analysis and excluded from the output in the analysis on PPI) will be included and flagged in the listings as results not included in the PPI analyses.

Key immunogenicity subgroup analyses will also be performed by age group, sex, BMI, race, ethnicity, and serostatus at baseline, as defined in Section 5.1.5.

Categorical variables will be summarized with a frequency table showing counts and percentages.

Continuous variables will be summarized using the following statistics, as appropriate: number of observations, geometric mean, arithmetic mean (mean), 95% confidence interval (CI) for the mean, standard deviation (SD), standard error (SE), median, quartiles (Q1 and Q3), minimum and maximum.

Data listings, participant profiles and/or participant narratives may be provided as appropriate.

Binary variables will be summarized using the following statistics: number of observations, percentages, and Exact Clopper-Pearson 95% CIs.

Formal NI testing will be applied to demonstrate NI of 1-dose and 2-doses of $9x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, $2.5x10^{10}$ vp and $1.25x10^{10}$ vp Ad26.COV2.S versus 1- dose and 2-doses of $5x10^{10}$ vp Ad26.COV2.S (release titer), using a NI margin of 2/3 for the GMC ratios.

The following 2 sets of hypotheses (1 dose and 2 doses) will independently be tested sequentially:

Post-dose 1

1a) NI after 1-dose of Ad26.COV2.S $9x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

1b) NI after 1-dose of Ad26.COV2.S $2.5x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

1c) NI after 1-dose of Ad26.COV2.S $7x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

1d) NI after 1-dose of Ad26.COV2.S $3.5x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

1e) NI after 1-dose of Ad26.COV2.S $1.25x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

In this set, NI will be demonstrated in terms of humoral immune response expressed by the GMCs of S-ELISA, 28 days post-dose 1, using a NI margin of 2/3 for the GMC ratio (GMC [$9x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, $2.5x10^{10}$ or $1.25x10^{10}$ vp]/GMC $5x10^{10}$ vp).

Post-dose 2

2a1) NI after 2-doses of Ad26.COV2.S $9x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2a2) NI after 2-doses of Ad26.COV2.S $9x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2b1) NI after 2-doses of Ad26.COV2.S $2.5x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2b2) NI after 2-doses of Ad26.COV2.S $2.5x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2c1) NI after 2-doses of Ad26.COV2.S $7x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2c2) NI after 2-doses of Ad26.COV2.S $7x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2d1) NI after 2-doses of Ad26.COV2.S $3.5x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2d2) NI after 2-doses of Ad26.COV2.S $3.5x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2e1) NI after 2-doses of Ad26.COV2.S $1.25x10^{10}$ vp versus 1-dose of Ad26.COV2.S $5x10^{10}$ vp (release titer)

2e2) NI after 2-doses of Ad26.COV2.S $1.25x10^{10}$ vp versus 2-doses of Ad26.COV2.S $5x10^{10}$ vp (release titer)

In this set, NI will be demonstrated in terms of humoral immune response expressed by the GMCs of S-ELISA, 14 days post-dose 2 ($9x10^{10}$ vp, $7x10^{10}$ vp, $3.5x10^{10}$ vp, $2.5x10^{10}$ vp or $1.25x10^{10}$ vp) and 28 days post-dose 1 or 14 days post-dose 2 ($5x10^{10}$ vp), using a NI margin of 2/3 for the GMC ratio (GMC [$9x10^{10}$ vp, $2.5x10^{10}$, $7x10^{10}$ vp, $3.5x10^{10}$ vp, or $1.25x10^{10}$ vp]/GMC $5x10^{10}$ vp

5.5.3.1. Non-Inferiority Analysis- Immune Response

Formal NI testing will be applied to demonstrate non-inferiority of immunogenicity. Non-inferiority will be analyzed by vaccine dose levels. As described in section 4, only one of the two scenarios will be considered for non-inferiority hypothesis testing at primary analysis. If there is an overall loss of > 40% evaluable data points in the NI set at Day 29 (defined as number of evaluable participants in the NI set at D29/total number of subjects in Table 4), the population in scenario 2 will be used for primary analysis.

- **Scenario 1 (base case scenario):** non-inferiority will be performed on the NI set including participants from the main and the sub study. A linear regression analysis with only one main effect (dose level) will be fitted, with log-transformed (log_{10} scale) SARS-CoV-2 S-protein specific antibody concentration (S-ELISA) as the response variable and dose level as the independent variable.

- **Scenario 2**: Non-inferiority will be performed on the PPI set including participants from the main study and sub-study. A linear regression analysis with two main effects (dose level and SARS-CoV-2 serostatus at baseline) will be fitted, with log-transformed (log_{10} scale) SARS-CoV-2 S-protein specific antibody concentration (S-ELISA) as the response variable and dose level and SARS-CoV-2 serostatus at baseline as the independent variables.

Participants with missing serostatus at baseline will not be included in this analysis.

Level of Significance

For each of the 1-dose and 2-dose vaccine regimens hypothesis, the type I error rate α will be set to 0.0125 (1-sided) and non-inferiority (NI) tests will be confirmed by constructing the corresponding 97.5% confidence interval (2-sided) which is equivalent to using $\alpha = 0.025$ (1-sided) with 98.75 (1-sided CI).

Multiplicity Adjustment

The family-wise error rate (FWER) at α = 0.025 (one-sided), will be controlled by:

Splitting the Type I error rate α = 0.025 into two (i.e., α = 0.0125) using Bonferroni correction for the independently tested 1 and 2 doses NI hypotheses (see

Figure 1).

Figure 1 depicts a tree-based schema for testing the non-inferiority hypotheses controlling the family-wise error rate (FWER) at α = 0.025 (one-sided). Non- inferiority of immunogenicity will be assessed by first constructing the 2-sided (1- α) % confidence interval for the difference in means based on the sampling distribution of two independent normal distributions with variances that are unknown but assumed equal.

Let $\boldsymbol{X_g = \log_{10} Y_g \sim N(\mu_g, \sigma_g)}$, normally distributed with true population mean and standard deviation, $\boldsymbol{\mu_g}$ and $\boldsymbol{\sigma_g}$ respectively and where $\boldsymbol{X_g}$ is the log-transformed ($\log_{10}$ scale) SARS-CoV-2 S-protein specific antibody concentration (ELISA) data for vaccine dose levels, $\boldsymbol{g = A, B, C, D, E}$ in each hypothesis test (so defined to reflect the hypotheses in

Figure 1 in section 1.3),

Where, A = $9\text{x}10^{10}$ vp; B = $2.5\text{x}10^{10}$ vp; C = $3.5\text{x}10^{10}$ vp; D = $7\text{x}10^{10}$ vp; E = $1.25\text{x}10^{10}$ vp

Let $X_r = \log_{10} Y_r \sim N(\mu_r, \sigma_r)$, where X_r is the log-transformed ($\log_{10}$ scale) SARS-CoV-2 S-protein specific antibody concentration (S-ELISA) data for vaccine dose level $5\text{x}10^{10}$ vp (release titer), the reference dose level, r.

It can be shown that the log-transformed ($\log_{10}$ scale) SARS-CoV-2 S-protein specific antibody concentration (S-ELISA) mean differences between the vaccine dose levels g, $\left(\overline{X_g} - \overline{X_r}\right)$, is equal to the log-transformed ($\log_{10}$ scale) ratio of the corresponding geometric means, i.e., $\log_{10}\left(GMC_g/GMC_r\right)$

Hence, back transforming yields, $GMC_g/GMC_r = 10^{\left(\overline{X_g} - \overline{X_r}\right)}$ as the geometric mean ratio estimates.

Next, we can construct the (1- α) % confidence interval (2-sided) for the geometric mean ratios, by first constructing the confidence interval on the difference on the means, $\left(\overline{X_g} - \overline{X_r}\right)$.

Assuming unknown equal variances for the population vaccine dose levels g and release titer, reference dose level, r, and log-transformed ($\log_{10}$ scale) SARS-CoV-2 S-protein specific antibody concentration (S-ELISA, the (1- α) % confidence interval (2-sided) estimate for the population vaccine dose level g and reference dose level r mean differences, is given by,

$$\left(\overline{X_g} - \overline{X_r}\right) \pm t_{(1-\alpha/2\,;v_p)} S_p \sqrt{\left(\frac{1}{n_g} + \frac{1}{n_r}\right)}$$

Where the pooled variance estimate, $S_p^2 = \frac{(n_g-1)S_g^2+(n_r-1)S_r^2}{n_g+n_r-2}$ and $v_p = n_g + n_r - 2$ is the degrees of freedom for the t-distribution and $\alpha = 0.0125$.

Since log transformation is monotone, back transforming confidence intervals for the difference in means in the log scale gives a confidence interval for the ratio of the geometric means. Hence, the lower and upper bounds of the 2-sided $(1-\alpha)\%$ confidence interval for the GMC ratios are as follows:

$$\left((GMC\ Ratio)\right)_{Lower\ Bound} = 10^{\left(\left(\overline{X_g}-\overline{X_r}\right) - t_{(1-\alpha/2\,;v_p)} S_p \sqrt{\left(\frac{1}{n_g}+\frac{1}{n_r}\right)}\right)}$$

$$\left((GMC\ Ratio)\right)_{Upper\ Bound} = 10^{\left(\left(\overline{X_g}-\overline{X_r}\right) + t_{(1-\alpha/2\,;v_p)} S_p \sqrt{\left(\frac{1}{n_g}+\frac{1}{n_r}\right)}\right)}$$

For each of the vaccine dose level, g comparisons (post-dose 1 and post-dose 2), NI will be demonstrated if

$$\left((GMC\ Ratio)\right)_{Lower\ Bound} > \frac{2}{3}$$

If the assumptions of the unknown equal variances for the independent normal distributions of vaccine dose levels do not hold (i.e., test of equality variances shows that the variances are significantly different), then an approximate t-distribution with Satterthwaite approximated degrees of freedom will be used instead of (6) (Casella, G & Berger L.R (1990).

SAS v 9.4 will be used in implementing the above NI comparisons.

Remarks:

No generally accepted immunological correlate of protection has been demonstrated for SARS-CoV-2 to date. If a correlate or threshold for protection against COVID-19 is established in terms of humoral immunity, then a statistical comparison to that correlate or threshold will be performed in addition, as outlined in a revised analytical plan. If the correlate or threshold of protection will be used as an endpoint the definition of seroconversion and seroconversion rate will be described in Section 5.7.

5.6. Secondary Humoral Immune Response Endpoints

5.6.1. Definition of Endpoint

The secondary humoral immune response endpoints are (at all blood collection timepoints):

Seroconversion is defined as participant test positive at any timepoint while the participant was seronegative at baseline.

Main study:

Serological response to vaccination and binding antibody GMCs to SARS-CoV-2 S protein as measured by ELISA, or equivalent assay.

Antibody GMCs (S-ELISA).

The proportion of participants achieving seroconversion of serum antibody against the SARS-CoV-2 S protein by S-ELISA

Sub study

Serological response to vaccination and binding antibody GMCs to SARS-CoV-2 S protein as measured by ELISA, or equivalent assay.

Antibody GMCs (S-ELISA)

The proportion of participants achieving seroconversion of serum antibody against the SARS-CoV-2 S protein by S-ELISA

GMCs derivations will be based on log-transformed (log_{10} scale) immune response (ELISA concentrations). This will be applied for both main study analysis and sub study analysis.

5.6.2. Estimand

Not applicable

5.6.3. Analysis Methods

Descriptive statistics (geometric mean and confidence intervals, or median and interquartile range Q1-Q3, as appropriate) will be calculated for all blood collection timepoints based on the log-transformed (log_{10} scale) immune response (ELISA concentrations) data. Geometric mean fold-increase in titers over baseline and the corresponding 95% CI in the S-ELISA will also be calculated. Geometric mean fold increases will be derived by first calculating the means of each blood collection timepoint change from baseline (pre-first vaccination or pre-second vaccination) log-transformed (log_{10} scale) immune response (ELISA concentrations) and then back-transforming to the original scale. Further analysis methods are described in Section 6.1.

5.7. Exploratory Analysis

Definitions and details about the analysis methods for exploratory humoral immunogenicity endpoints are given in Section 6.1.

Main study:

Exploratory analyses may be performed at the request of the sponsor on a subset of participants. If the subset selection is to be performed prior to primary analysis and is potentially unblinding, the selection will be carried out by an external unblinded party.

The following exploratory humoral immune responses to Ad26.COV2.S may be performed on a subset of participants:

- SARS-CoV-2 neutralization as assessed by SARS-CoV-2 neutralization assays VNA (wtVNA and/or psVNA) including neutralization of emerging SARS-CoV-2 variants.
- SARS-CoV-2 binding antibodies against emerging SARS-CoV-2 variants as assessed by S-ELISA or equivalent assay Adenovirus neutralization.
- Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, immunoglobulin (Ig) subclass and IgG isotype.
- Analysis of circulating Spike protein
- Epitope-specificity characterization of antibodies.
- Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in the serum or plasma.
- Passive transfer: Analysis of immune mediators correlating with protection against experimental SARS-CoV-2 challenge in a suitable animal model.

Correlations include but are not limited to :

- Correlation between ELISA (S-ELISA; EU/mL) and VNA (wild-type virus [wt]VNA and/or pseudovirion [ps]VNA, including emerging variants) titers at selected timepoints.
- Correlation between Adenovirus neutralization (Ad26 VNA) and S-ELISA or VNA(wtVNA and /or psVNA, including emerging variants) titers at selected timepoints

Occurrence of asymptomatic SARS-CoV-2 infection.

- The number of participants with positive non-S protein ELISA or equivalent assay (eg, nucleocapsid [N] protein ELISA).

Immune response in vaccinated individuals with prior or breakthrough infection.

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

Sub study

Humoral response to Ad26.COV2.S may include the following

- SARS-CoV-2 neutralization as assessed by SARS-CoV-2 neutralization assays VNA (wtVNA and/or psVNA) including neutralization of emerging SARS-CoV-2 variants
- SARS-CoV-2 binding antibodies against emerging SARS-CoV-2 variants as assessed by S-ELISA or equivalent assay
- Adenovirus neutralization
- Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, immunoglobulin (Ig) subclass and IgG isotype
- Analysis of circulating Spike protein
- Epitope-specificity characterization of antibodies
- Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in the serum or plasma.

Correlations include but are not limited to:

- Correlation between ELISA (S-ELISA; EU/mL) and VNA (wild-type virus [wt]VNA and/or pseudovirion [ps]VNA, including merging variants) titers at selected timepoint.
- Correlation between Adenovirus neutralization (Ad26 VNA) and S-ELISA or VNA (wtVNA and /or psVNA, including emerging variants) titers at selected time points.

Occurrence of asymptomatic SARS-CoV-2 infection.

- The number of participants with positive non-S protein ELISA or equivalent assay (eg, nucleocapsid [N] protein ELISA)

Immune response in vaccinated individuals with prior or breakthrough infection

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

To evaluate the innate, pro-inflammatory and other potentially relevant responses to Ad26.COV2.S vaccination at selected timepoints.

- Analysis of messenger ribonucleic acid (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.

6. OTHER ANALYSES

6.1. Immunogenicity Analyses

Immunogenicity analyses will use the PPI and/or NI set (as applicable). Where relevant, immunogenicity analyses will include SARS-CoV-2 serostatus at baseline as a subgroup. Immunogenicity analyses will also be done on the FAS (participants who became infected during

the study will be analyzed as a subgroup and shown in the graphs using different colors and symbols). Data will be analyzed by vaccine group.

Key immunogenicity assay results will also be analyzed for the subgroups defined in Section 5.2.1.

Data will be presented by scheduled time point. For the PPI analysis, samples taken outside of the allowed window as described in section 5.1.3 will be excluded from the tables and graphs (but will be included in the listings and clearly marked as results not included in the PPI analyses). For the FAS analysis, samples taken outside of the allowed window will be included.

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

The following exploratory analysis will be performed on the sub-study for descriptive purposes:

- Transcriptomics: The relative quantity of transcripts (as measured by mRNA sequencing) in whole blood following vaccination will be measured. The magnitude and type of differentially expressed genes relative to baseline and their kinetics will be compared post dose 1 and post dose 2, and between dose levels. Details are described in separate a study plan and Omics Statisticial Analysis Plan.
- Proteomics: The relative abundance of serum proteins following the first and second dose of different Ad26.COV2.S dose levels will be measured relative to baseline and compared between dose levels. Details are described in a separate study plan and Omics Statistical Analysis Plan.
- Circulating spike: The concentration of circulating Spike protein over time in participants who received selected Ad26.COV2.S dose levels will be measured and compared between doses. Details will be described in a separate study plan.

6.1.1. Parameters

The following humoral immune responses may be measured. However, not all assays might be available for all immunogenicity analyses covered by this SAP. Further information on which assays will be analyzed in each of the analyses, will be included in the corresponding DPS documents.

Table 7: Summary of Humoral Immunogenicity Assays

Assay	Purpose
Humoral Immunogenicity	
Primary/Secondary/Exploratory endpoints	
SARS-CoV-2 binding antibodies (ELISA)	Analysis of antibodies binding to SARS-CoV-2 or individual SARS-CoV-2 proteins (eg, S protein)
Exploratory endpoints	
SARS-CoV-2 neutralization (VNA)	Analysis of neutralizing antibodies to the wild-type virus and/or pseudovirion expressing S protein, including emerging SARS-CoV-2 variants
SARS-CoV-2 binding antibodies (ELISA or equivalent assay)	Analysis of binding antibodies to SARS-CoV-2 proteins (eg, S-protein), including emerging SARS-CoV-2 variant proteins.
SARS-CoV-2 binding antibodies (non-S-ELISA and/or SARS-CoV-2 immunoglobulin assay)	Analysis of antibodies binding to the SARS-CoV-2 N protein
Adenovirus neutralization	Adenovirus neutralization assay to evaluate neutralizing antibody responses against the Ad26 vector
Functional and molecular antibody characterization	Analysis of antibody characteristics including Fc-mediated viral clearance, avidity, Fc characteristics, Ig subclass and IgG isotype
Epitope-specificity characterization	Analysis of site-specificity, epitope mapping
Cytokine profiling, and analysis of circulating Spike protein	Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in the serum or plasma, and analysis of circulating Spike protein.
Passive transfer	Analysis of immune mediators correlating with protection against experimental SARS-CoV-2 challenge in a suitable animal model
Innate Assessments (Exploratory endpoints)	
Gene expression analysis	Analysis of gene expression by RNA transcript profiling in whole blood (ex vivo, PaxGene tubes)
Proteomic and/or lipidomic approaches, and analysis of circulating Spike protein	Analysis of protein translates (including circulating Spike protein) or lipid mediators in serum or plasma.

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

6.1.2. Handling of Missing and/or Unquantifiable Immune Response Data

1. Missing immune response data will not be imputed.

2. Values below the lower limit of quantification (LLOQ) or limit of detection (LOD) will be handled as follows:

   - Calculation of geomean and median:

- Values < LLOQ are imputed with LLOQ/2

For Ad26 VNA,

- Values < LOD are imputed with LOD/2
- Values between LLOQ and LOD are imputes with (LLOQ + LOD) /2

- Calculation of fold increases from baseline:
  - values < LLOQ are imputed with LLOQ

For Ad26 VNA,

- Values < LOD are imputed with LOD
- Values between LLOQ and LOD are imputes with (LLOQ + LOD) /2

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

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

If >10% of data points in PPI or NI Set data values are > ULOQ, a selection of descriptive immunogenicity assessments will be repeated without a ULOQ.

6.1.3. Handling of Changes in Assay Status Throughout the Study Conduct

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

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

6.1.4. Humoral Assays

For **S-ELISA** and **VNA** assay **(**both wild-type virus and pseudo-virion expressing S protein, for the original strain but also on the emerging variants as available), the following results will be calculated: N, geometric mean[1§] and corresponding 95% CI of the actual values and fold increases from baseline, fold increase from pre-dose 2 (for time points after dose 2) will be tabulated and graphically presented.

This will be applied for both main study analysis and sub study analysis.

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

For S-ELISA, wild type, and pseudo-virion VNA (including emerging variants) separately:

- A sample will be considered positive if its value is strictly > LLOQ.
- The following responder definition (Janssen responder definition) will be used as the core definition to assess sero-response rates. A post-baseline sample will be considered a responder if at least one of the following conditions is satisfied.
- The baseline (pre-dose 1) sample value is ≤ LLOQ and the post-baseline sample is strictly > LLOQ
- The baseline (pre-dose 1) sample value is strictly greater than the LLOQ (> LLOQ) and the post-baseline sample value represents an at least 4-fold (≥ 4-fold) increase from the baseline sample value.

An alternative responder definition (definition requested by FDA for other studies) may be used for exploratory purposes only to assess sero-response rate in addition to the one above. In such cases, separate tables including sero-response rates with this alternative responder definition will be generated. Under the FDA responder definition, a post-baseline sample will be considered a responder if at least one of the following conditions is satisfied:

- The baseline (pre-dose 1) sample value is < LLOQ and the post-baseline sample is ≥ 4x LLOQ.
- The baseline sample (pre-dose 1) value is > LLOQ and the post-baseline sample value represents a ≥ 4-fold increase from the baseline sample value.

Actual values are tabulated and shown as dot plots with dots for participant values, and the corresponding geometric mean and 95% CI per time point for each assay. In addition, GMC plots over time, combining the regimens in one graph (without individual participant dots) will also be generated.

- Participant profiles of the actual values over time will be graphically presented.
- Reverse distribution curved of the actual values are provided for selected time points.
- In the graphs, original values will be displayed in the $\log_{10}$ scale.

- Further details and/or updated rules or graphs will be provided in the DPS.

The ratio of binding antibodies (S-ELISA) to wild type VNA, and the ratio of binding antibodies (S-ELISA) to pseudo-virion expressing S protein VNA (including emerging variants) will be calculated for each time point. Values < LLOQ will be imputed with LLOQ for the calculation of the ratios.

In addition, the ratio of the fold increase from baseline in binding antibodies (S-ELISA) to the fold increase from baseline in wild type VNA, and the ratio of the fold increase from baseline in binding antibodies (S-ELISA) to the fold increase from baseline in pseudo-virion expressing S protein VNA will be calculated for each post-baseline time point. Values < LLOQ will be imputed with LLOQ for the calculation of the fold increase ratios.

The following statistics will be calculated and tabulated: N, geometric mean and corresponding 95% CI of the ratio. Graphical displays will also be prepared, showing – for each time point – the geometric mean of the ratio and its 95% CI, together with the individual data points (dot plot).

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

Correlation (at selected timepoints)

The correlation between the binding antibody (ELISA) titers and neutralizing antibody (VNA) titers to SARS-COV-2 will be assessed in a subset of participants at selected timepoints using spearman's rank correlation. ELISA (S-ELISA; EU/mL) and VNA (wtVNA or psVNA including emerging variants; IC50) titers. This will be applied for both main study analysis and sub study analysis, as available.

Adenovirus 26 neutralization will be measured in a subset of participants at Day 1 and at Day 57 to assess the impact of pre-existing Ad26 neutralizing antibodies in humoral immunogenicity against SARS-CoV-2 (S-ELISA, psVNA including emerging variants and/or wtVNA). Ad26 VNA sample values >LOD are considered positive.

Descriptive statistics (N, geometric mean and corresponding 95% CI, number, and percentage of positive samples) will be calculated. Spearman correlation between Ad26 VNA at Day 1 (if sufficient number of Ad26-seropositive participants) or Day 57 and S-ELISA concentrations or psVNA including emerging variants or wtVNA titers at Day 29 or Day 71, respectively, will be calculated and visualized with a scatterplot.

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

Binding antibodies (S-ELISA) versus wild type VNA

Binding antibodies (S-ELISA) versus pseudo-virion expressing S protein VNA (including emerging variants)

Wild type VNA versus pseudo-virion expressing S protein VNA

Ad26 VNA versus binding antibodies (S-ELISA)

Ad26 VNA versus wild-type VNA

Ad26 VNA versus pseudo-virion expressing S protein VNA (including emerging variants)

If a similar assay is performed at different analyzing labs, then the statistical analyses may distinguish between these and provide separate scatterplots for each analyzing lab versus the other assay of interest. These scatterplots will display the values as analyzed for the geometric mean calculations. Spearman's rank correlation coefficients will also be provided (one per scatterplot).

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

Definition of SARS-CoV-2 infections

A SARS-CoV-2 infection is defined as a participant with:

- serologic conversion between baseline (Day 1; pre-vaccination) and selected timepoints post-vaccination using an ELISA and/or SARS-CoV-2 immunoglobulin assay that is dependent on the SARS-CoV-2 N protein (e.g., N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay), for participants that are N serology negative at baseline, or
- reported positive SARS-CoV-2 PCR test during the study.

For participants with missing N serology result at baseline, a post baseline positive N serology result will not be considered a COVID-19 infection.

Assessment of occurrence of asymptomatic SARS-CoV-2 infection

Serologic conversion between baseline (Day 1; pre-vaccination) and selected timepoints post-vaccination using an ELISA and/or SARS-CoV-2 immunoglobulin assay that is dependent on the SARS-CoV-2 N protein (e.g., N-ELISA or equivalent assay, such as the Roche Elecsys anti-SARS-CoV-2 assay), for participants that are N serology negative at baseline.

The proportion of participants who serologically converted will be graphically visualized and tabulated at every available timepoint by randomized group, together with the numbers of participants with an available measurement.

The number of participants with missing baseline N serology results will also be summarized.

Additional tabulation will summarize the number of participants with and without a SARS-CoV-2 infection as having:

Not infected

Asymptomatic or undetected participants

Antibodies binding to the SARS-CoV-2 nucleocapsid (N) protein

For antibodies binding to the SARS-CoV-2 nucleocapsid (N) protein, the number and percentage of participants with a positive sample at Day 1, Day 29, Day 71, Week 32 and Week 60 (if applicable) will be tabulated. A positive or negative result will be obtained from the assay (qualitative assay).

In contrast to the other immunogenicity analyses, this analysis will be performed only on the FAS.

The definition of the PPI analysis set incorporates the N-serology sample positivity assessment: immunogenicity samples obtained after a positive N-serology result post baseline will be excluded from the PPI analysis if the baseline N serology result was negative, as this is indicative of a SARS-CoV-2 infection during the study.

7. PLANNED ANALYSIS

The sponsor may be unblinded for this study, but the blind will be maintained at the participant and study site level up to study end.

The primary analysis, which will be performed on unblinded data, will include safety up to at least Day 85 and immunogenicity up to Day 71 in the main study and sub study for all groups and will be performed when all participants have completed the visit that takes place 85 days after the first study vaccination or discontinued earlier.

The final analysis for the main study will be performed when all included participants from the main study have completed their last visit (at least 6 months post last vaccination) or discontinued earlier. Results from participants in the sub study may be available with the main study. Results from the common analyses for then main study and sub study will be combined in one report while sub-study specific analysis will have a different report.

8. INDEPENDENT DATA MONITORING COMMITTEE (IDMC)

An Independent Data Review Committee (IDMC) has been commissioned to review the safety data of this trial alongside the other COVID-19 trials. Please refer to the IDMC Charter. Any relevant safety information from this study will be shared with the IDMC.

9. SUPPORTING DOCUMENTATION

9.1. Appendix 1: List of Abbreviations

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

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

9.2. Appendix 2: 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 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 judgment when characterizing bradycardia among some healthy participant populations, for example, conditioned athletes.
[#] Revised by the sponsor.

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

Myalgia[#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities; Use of narcotic pain reliever	Hospitalization; Inability to perform basic self-care functions

[#] Revised by the sponsor.

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

[#] Revised by the sponsor.

B: Tables for Laboratory Abnormalities

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

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

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen	23 – 26	27 – 31	> 31	Requires dialysis

(BUN) mg/dL				
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 –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

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

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as potentially life-threatening (Grade 4). For example. a low sodium value that falls within a Grade 3 parameter (125-129 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	Hyper-eosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

* 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. REFERENCES

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