Statistical Analysis Plan

ABNCoV2-01

An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects

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Signature Page

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Version History

The included analyses described are based on the final clinical trial protocol *An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects Edition 3.0,* dated 15 October 2021.

SAP Version	Date	Change	Rationale
1.0	16-Dec-2021	N/A	Original

1 Introduction

This Statistical Analysis Plan (SAP) details the statistical methodology to be used in analyzing trial data and outlines the key statistical programming specifications. It describes the variables and populations, anticipated data transformations and manipulations, and other details of the analyses not provided in the trial protocol. The SAP is written based on recommendations from *ICH E3: Structure and Content of Clinical Study Reports*, and *ICH E9: Statistical Principles for Clinical Trials*. Table, figure and listing shells are described in a separate document.

This SAP will be followed completely for the analysis of data derived from the clinical trial. Changes to the protocol planned analyses are described in Section 4.7. If any unforeseen additional analyses are included in the clinical study report (CSR) they will be clearly described as additional, unplanned analyses in Section 4.7 if added prior to database lock, or to the equivalent section of the CSR if ad-hoc analyses are requested post-database lock.

1.1 Objectives, Endpoints, and Estimands

This is a phase 2 trial of adult subjects who are seropositive or seronegative for SARS-CoV-2 at baseline. Immunogenicity, tolerability, and safety data will be included in the analysis and CSR for this trial.

Objectives	Endpoints
Primary	
To assess SARS-CoV-2 specific humoral immune responses of the ABNCoV2 vaccine in initially SARS-CoV-2 seronegative and seropositive subjects.	Primary: SARS-CoV-2 neutralizing antibody titers at 2 weeks after the last vaccination, i.e., after the second vaccination in initially seronegative subjects (Group 1) and after the single boost vaccination in initially seropositive subjects (Groups 2 and 3), for subjects in the Immunogenicity Analysis Set (Section 4.1.2). Sensitivity Analyses: SARS-CoV-2 neutralizing antibody titers at 2 weeks after the last vaccination for all vaccinated subjects (Safety

Objectives	Endpoints
	Analysis Set) with at least one post-vaccination neutralizing antibody titer as an observed case analysis. Additionally, a multiple imputation analysis will be performed using the Safety Analysis Set for a full accounting of the trial population. Supportive Endpoints: SARS-CoV-2 neutralizing antibody response, defined as achieving SARS-CoV-2 neutralizing antibody titers above the limit of quantification in Group 1, or having an at least a 2-fold or 4-fold increase from baseline in Groups 2 and 3, respectively, for subjects in the Immunogenicity Analysis Set.
Secondary	
To assess the safety and tolerability of the ABNCoV2 vaccine in adult seropositive and seronegative subjects.	Secondary: • Subjects reporting any Serious Adverse Events (SAEs) or Adverse Events of Special Interest (AESIs) assessed as related to trial vaccine within 8 days after vaccination • Subjects reporting any Grade 3 or higher Adverse Events (AEs) assessed as related to trial vaccine within 8 days after vaccination • For the primary analysis of these endpoints, if the investigator confirms the subject discontinues the trial within 8 days after vaccination due to AEs, the subject is considered having a related SAE/AESI/Grade 3 or higher AE regardless of the seriousness, severity, or causality of the AE for which the subject discontinued. This is consistent with the "composite" strategy. Sensitivity Analyses: • Subjects who discontinue the trial within 8 days after vaccination due to AE will be analyzed using the "treatment policy" strategy to handle the intercurrent event, where the subject will not be considered having a related SAE/AESI/Grade 3 or higher AE assessed as related to trial vaccine, unless the specified AE leading to discontinuation meets one or more of these categories.

Objectives	Endpoints
	Supportive Endpoints: • Subjects reporting any SAE or AESI, regardless of relationship, within 29 days after vaccination. • Subjects reporting any Grade 3 or higher AEs assessed as related to trial vaccine within 29 days after vaccination. • Subjects reporting solicited local AEs within 8 days after vaccination. • Subjects reporting solicited general AEs within 8 days after vaccination.
Exploratory	
1. To assess SARS-CoV-2 specific peak humoral immune responses after the prime-boost regimen (Group 1) or the booster dose (Group 2 and Group 3) of the ABNCoV2 vaccine. 2. To explore SARS-CoV-2 specific cellular immune responses to the ABNCoV2 vaccine. 3. To assess SARS-CoV-2 specific neutralizing immune responses against variant strains circulating at the time of analysis.	1. a) SARS-CoV-2 neutralizing antibody titers at 2 and 4 weeks post-prime vaccination and 1, 4, and 13 weeks post-boost vaccination (Group 1), and 1 week, 4 weeks, 3 months, 6 months, 1 year and 2 years after vaccination (Groups 2 and 3). b) Frequency and percentage of subjects in Groups 2 and 3 with SARS-CoV-2 neutralizing antibody titers having an at least 2-fold increase and 4-fold increase, respectively, from baseline at each sampling time point. c) SARS-CoV-2 total antibody titers (by ELISA) at all sampling time points. 2. Cellular immune response, by Interferon-γ / Interleukin-4 ELISPOT, at 1 week after the last vaccination for all subjects. 3. SARS-CoV-2 neutralizing antibody titers against variant strains circulating at the time of analysis at 2 weeks after last vaccination for all subjects.

Primary Estimand

The primary clinical question of interest is: What peak SARS-CoV-2 neutralizing antibodies titers can be achieved in seronegative (Group 1) and seropositive (Groups 2 and 3) adult subjects after protocol-specified vaccination regimen with ABNCoV2?

Treatment Condition: open-label, non-randomized treatment where subjects follow the protocol requirements and have no deviations from the protocol which would affect their SARS-CoV-2 neutralizing antibody titer levels at two weeks post the last vaccination with ABNCoV2.

Population: Seronegative (Group 1) or seropositive (Groups 2 and 3) adult subjects who meet all of the inclusion and none of the exclusion criteria for the trial, have their 2 weeks post-last vaccination neutralizing antibody titer result, and have no protocol violations which would affect the immunogenicity assessment for the trial.

Endpoint: SARS-CoV-2 neutralizing antibody titer at 2 weeks post-last vaccination (second vaccination for Group 1, and single boost vaccination for Groups 2 and 3).

Intercurrent Events:

1. Development of COVID-19 symptoms prior to 2 weeks after the last vaccination (Week 6 in Group 1 and Week 2 in Groups 2 and 3) and having a positive test for SARS-CoV-2 infection.

   Note, subjects who come in contact with the virus can have significant interference with the SARS-CoV-2 neutralizing antibody levels and thus will be excluded from the primary analysis. This is equivalent to the "while on treatment" strategy.

2. Discontinuation due to AEs from either the second vaccination (Group 1) or the trial (all groups).

   Note, for the primary estimand the second intercurrent event is not considered as having an impact on the SARS-CoV-2 neutralizing antibody levels and therefore the "treatment policy" strategy will be used.

Population-level summary:

Group 1 - Geometric mean of SARS-CoV-2 neutralizing antibody titers at 2 weeks after the second vaccination.

Groups 2 and 3 - Geometric mean of the fold increases, defined as the ratio of SARS-CoV-2 neutralizing antibody titer at 2 weeks post single boost vaccination divided by the neutralizing antibody titer at baseline (last measurement prior to ABNCoV2 vaccination).

Rationale for estimand: The primary estimand will allow Bavarian Nordic (BN) in this early phase trial to understand the immunogenic effect of the ABNCoV2 vaccine in subjects treated per protocol specifications to inform future later phase trials.

Secondary Estimands

The secondary clinical questions of interest are:

- What percentage of subjects experience an SAE or AESI assessed as related to trial vaccination within 8 days after vaccination?

- What percentage of subjects experience a Grade 3 or higher AE assessed as related to trial vaccination within 8 days after vaccination?

Treatment Condition: open-label, non-randomized treatment where subjects receive at least one vaccination.

Population: Seronegative (Group 1) or seropositive (Groups 2 and 3) adult subjects who meet all of the inclusion and none of the exclusion criteria for the trial, and have received at least one trial vaccination.

Endpoints: Occurrence of a related SAE, AESI, or Grade 3 or higher AE within 8 days after vaccination.

Intercurrent Events: Discontinuation due to AEs from either the second vaccination (Group 1) or the trial (all groups). If it is confirmed by the investigator that the subject discontinues the trial within 8 days after vaccination due to AEs, the subject is considered having a related SAE/AESI/Grade 3 or higher AE regardless of the seriousness, severity, or causality of the AE for which the subject discontinued. This is consistent with the "composite" strategy.

Note, including subjects who withdraw due to AEs within 8 days after vaccination as having had an SAE/AESI/Grade 3 or higher AE regardless of the seriousness, severity, or causality of the AE for which the subject discontinued will only occur for these secondary endpoints. The remaining summaries for safety will be presented as collected.

Population-level summary: Percent of subjects reporting any SAE or AESI assessed as related to trial vaccine within 8 days after vaccination, and percent of subjects reporting any Grade 3 or higher AE assessed as related to trial vaccine within 8 days after vaccination.

1.2 Trial Design

- Open label
- Phase 2
- Safety, tolerability, and immunogenicity trial
- Non-randomized
- 210 healthy, adult volunteers grouped as follows:
    - Group 1: Approx. 30 subjects determined to be seronegative for SARSCoV-2 antibodies at screening (100 µg dose).
    - Group 2: Approx. 90 subjects determined to be seropositive for SARS-CoV2 antibodies at screening (100 µg dose).
    - Group 3: Approx. 90 subjects determined to be seropositive for SARS-CoV2 antibodies at screening (50 µg dose).

For this trial, in a run-in phase 6 adults (comprising of 3 subjects in each Group 1 and 2) will be vaccinated at 1 clinical trial site in a consecutive manner, with an at least 48 hours interval between the first and second subject of each group, then the third subjects dosed on consecutive days, before opening up to full enrollment of the trial. Safety assessments will be based on

solicited and unsolicited AE data through the first week after first vaccination for all 6 subjects, evaluated by an independent Data Monitoring Committee (DMC). After a positive DMC recommendation, enrollment to the rest of Groups 1 and 2 of the trial will commence. Group 3 subjects will be enrolled after completion of Group 2 enrollment. The total duration of subject participation in the trial will be up to 104 weeks (Groups 2 and 3, one-dose vaccination schedule) or 17 weeks (Group 1, two-dose vaccination schedule). The duration of the trial as a whole ultimately depends on the length of the recruitment period.

A temporary halting or termination for the trial as a whole can be decided in case of:

- an SAE and serious AESI with an at least reasonable possibility of a causal relationship to the administration of ABNCoV2 vaccine
- an unexpected Grade 3 or higher systemic reaction or lab toxicity with at least a reasonable possibility of a causal relationship to the administration of ABNCoV2 vaccine

These parameters are not all-inclusive. Other AEs could occur that would trigger a DMC review. Any member of the DMC, the Principal Investigator (PI) and/or the BN Medical Monitor could request a DMC review based on any observation.

Additional immunogenicity sample collection will be performed at 3 months after the last vaccination for all groups, and 6 months, 1 year, and 2 years for Groups 2 and 3 subjects. SARS-CoV-2 infection testing will be performed through the Active Trial Period if clinically indicated.

The schedule of events for Group 1 is in Appendix 2 and for Groups 2 and 3 is in Appendix 3.

2 Statistical Hypotheses

2.1 Multiplicity Adjustment

As no formal hypothesis testing will be performed, no adjustments for multiplicity will be needed.

3 Analysis Sets

The target analysis population for the trial includes subjects who meet all of the inclusion and none of the exclusion criteria as defined separately for each group (see Protocol Section 1.3). Subjects in Group 1 will be confirmed to be seronegative, and subjects in Groups 2 and 3 will be confirmed to be seropositive, and be stratified for enrollment by reason for seropositivity such that at least 40 subjects over both groups are enrolled for each of the three reasons for seropositivity (prior COVID-19 infection, prior mRNA vaccination, prior Adenovirus-based vaccination).

For the purposes of analysis, the following analysis sets are defined in Table 1.

Table 1 Analysis Set Definitions

Participant Analysis Set	Description
Safety Analysis Set	All subjects who received at least 1 dose of ABNCoV2 vaccine. Subjects who are vaccinated are included in this set regardless of protocol violations or SARS-CoV-2 infection during the trial.
Immunogenicity Analysis Set	All subjects who are in the Safety Analysis Set and have at least 1 post-vaccination neutralizing antibody titer result. Subjects with protocol violations substantially affecting the immunogenicity outcomes will be excluded from this analysis set. For the purpose of the primary estimand, this includes SARS-CoV-2 infection prior to 2 weeks post last vaccination.

Protocol violations which may exclude a subject from the Immunogenicity Analysis Set may include, but are not limited to:

1. For Group 1, discontinuation prior to receiving both vaccinations
2. Enrollment into the incorrect SARS-CoV-2 exposure group, i.e., a seropositive subject enrolled into Group 1 or a seronegative subject enrolled into Group 2 or 3.
3. Major violation of the visit window affecting the immunogenicity results (i.e., the 2 weeks post-last vaccination serum sample is out of window).
4. Prohibited medication or vaccination given too close to the date of vaccination, or prior to collection of immunogenicity sample for the primary estimand time point, that may affect the immunogenicity results.
5. Major immunogenicity sample handling deviations at critical visits, e.g. regarding clotting time, turnaround time.
6. SARS-CoV-2 symptoms or infection prior to collection of the 2-week post-last vaccination immunogenicity sample.
7. Deviations with regard to vaccine preparation or administration.

Protocol violations leading to exclusion from the Immunogenicity Analysis Set will be reviewed on an ongoing basis, including reviews of the case report form data and protocol deviations as stored in the clinical trials management system. The final list of exclusions from the Immunogenicity Analysis Set will be approved at a data review meeting prior to the primary analysis reporting.

The following analysis sets will be used to obtain estimates of the primary and secondary estimands defined in the protocol, additional immunogenicity endpoints, and safety endpoints.

Table 2 Analysis Sets by Objective

Objectives	Analysis Sets
Analysis set for the primary estimand, supportive immunogenicity, and exploratory objectives	Immunogenicity Analysis Set Sensitivity for primary estimand: Safety Analysis Set with at least one post-baseline neutralizing titer, regardless of protocol deviations substantially affecting immunogenicity outcomes
Analysis set for secondary estimands and additional safety endpoints	Safety Analysis Set

4 Statistical Analyses

4.1 General Considerations

All individual data entered in the case report form will be listed as measured in subject-level data listings. Listings will be sorted by group, subject, time point, and parameter (if applicable), based on the domain presented. Tables for Group 1 will be created separately from Groups 2 and 3.

Subject population summaries including disposition, protocol deviations, demographics, medical history and baseline signs and symptoms, and prior and concomitant medications will be presented separately for seropositive and seronegative subjects:

Seropositive Table Columns

- Group 2
- Group 3
- Overall

Seronegative Table Column

- Group 1

For reporting of results collected at multiple visits (i.e., immunogenicity, vital signs, and laboratory measures) tables will be presented by time point and parameter.

In particular, Groups 2 and 3 tables for humoral immunogenicity endpoints will be presented with groupings based on each subject's baseline Wuhan strain neutralizing antibody titer value. The median of the values at or above the lower limit of quantitation (LLOQ) will be calculated from the pooled baseline values for Groups 2 and 3 data such that comparisons between dose levels and baseline neutralizing titer groups can be made. The following columns will be presented within each group:

- Overall
- Baseline NT <LLOQ

- Baseline NT ≥LLOQ
- Baseline NT ≥LLOQ - <Median
- Baseline NT ≥Median

Safety and secondary endpoint tables will be presented by group, without respect to baseline neutralizing antibody titer measure. Group 1 AE tables will be presented by vaccination period and for the Active Trial Period as a whole. Group 2 and 3 AE tables will be presented for the Active Trial Period (see Section 4.1.3). AEs will be summarized at both the event and subject level, with percentages based on subject counts.

With the exception of immunogenicity data, continuous measurements will be summarized using the number of observations (n), mean, standard deviation (SD), minimum (Min), median, and maximum (Max). Categorical data will be summarized using frequencies and percentages, unless otherwise stated.

Immunogenicity data which are log-normally distributed will be presented using n, geometric means and corresponding 95% confidence intervals (CI), median, Min, and Max. Figures will be scaled using a logarithmic y-axis.

Neutralizing antibody titers for the Wuhan strain will be scaled to WHO standard units (IU/mL), however neutralizing antibody titers for the variants of concern (e.g., alpha, beta, delta, and omicron strains) will be summarized as titer values. For all neutralizing antibody titer results, values below the lower limit of detection (LLOD) will be given a value of half value of the LLOD. Values that are below the LLOQ but at or above the LLOD will be analyzed as half the value of the LLOQ.

Total antibody titers by ELISA will also be scaled to WHO standard units (BAU/mL). Values below the LLOQ will be given a value of half of the LLOQ.

ELISPOT results will be presented as measured in spot forming units (SFUs)/10^6 PBMCs. Interferon-γ and Interleukin-4 results will be summarized separately within individual stimulant pools. Values below the LLOD will be given a value of half value of the LLOD. Values that are below the LLOQ but at or above the LLOD will be analyzed as half the value of the LLOQ. Values above the upper limit of quantitation (ULOQ) will be given a value of twice the ULOQ.

No statistical testing will be performed for the trial. All statistical summaries and analyses will be performed using SAS® version 9.4 or higher (SAS Institute, Cary, NC, USA).

4.1.1 Baseline

Baseline is defined as the last available measure prior to vaccination with ABNCoV2 (first vaccination for Group 1, single boost vaccination for Groups 2 and 3). Baseline values may come from the screening, first vaccination visit, or an unscheduled visit prior to the first

vaccination visit. If multiple pre-vaccination values are available, the one closest in time to the ABNCoV2 vaccination will be used as the baseline value. In the event that no time is collected for a value measured on the date of first vaccination, measurements required to be collected prior to vaccination per the clinical trial protocol will still be considered eligible for inclusion as baseline measurements.

Subjects missing baseline values will not be included in change/shift from baseline or ratio to baseline analyses, including the Groups 2 and 3 analyses of the primary estimand.

4.1.2 Analysis Sets

In general, the Immunogenicity Analysis Set will be used for analyses of immunogenicity. The Safety Analysis Set will be used for sensitivity immunogenicity analyses, secondary endpoint analyses, as well as all safety, demographics, and disposition analyses. For further details, see Section 3.

4.1.3 Study Periods and Windowing

The following study periods are defined for this trial:

Screening Period: The period from the subject's signing of informed consent to the trial through the date and time of first vaccination. AEs starting during this period are considered Baseline Signs and Symptoms.

Active Trial Period: The period from the first vaccination up to and including week 4 (28-35 days) after receiving their vaccination. A visit at the end of the Active Trial Period is scheduled per protocol. In the event of early withdrawal of a subject from the trial during the Active Trial Period, the EAP visit may occur prior to the 28-35 days post-last vaccination window. For the purpose of safety analyses, any safety information collected within 35 days of their vaccination received will be included in summaries of the Active Trial Period.

FU Period: The period from the EAP visit through the final follow up visit. Subjects have 3 FU visits at 4, 9 and 22 weeks after the scheduled EAP visit (Weeks 8, 13 and 26). Subjects may withdraw from treatment early but continue into the FU Period.

For assigning prior and concomitant medications, those ending prior to the first vaccination are considered prior medications. All other medications are considered concomitant medications.

For AEs and medications with partial or missing start and/or stop dates, the "worst case" should be applied to be conservative. Therefore, the following rules will apply:

AEs

- If start date is partial and any portion (i.e., year or month and year) would confirm the AE was prior to first vaccination, the AE will be considered a Baseline Sign or Symptom.
- If the start date is completely missing and the end date, or any portion of a partial end date, confirms the AE ended prior to first vaccination, the AE will be considered a Baseline Sign or Symptom.
- Otherwise, the AE will be considered as belonging to the Active Trial Period.

Medications

- If the end date is partial, and any portion confirms the medication was ended prior to first vaccination, the medication will be considered a Prior Medication.
- Otherwise, the medication will be considered a Concomitant Medication.

Per protocol, all visits are to occur within the specified window in the Trial Schedules (Appendix 2 and Appendix 3). For the purpose of the primary estimand, rules for inclusion into the Immunogenicity Analysis Set will ensure immunogenicity samples taken too far out of window will not be included for analysis. For sensitivity analyses, immunogenicity samples taken out of window will be handled as described in Section 4.2.3.1.

In general, safety analyses will be performed for the Active Trial Period, accounting for any observations between the first vaccination and the EAP visit, inclusive of the EAP visit and any unscheduled visits during this period. In the event of early discontinuation from the Active Trial Period (i.e., EAP occurs prior to one month post last vaccination), safety results through 35 days (upper limit of the EAP visit window) post-last vaccination will be analyzed along with the Active Trial Period summaries. For Group 1, safety summaries will be performed by vaccination as well as for the overall Active Trial Period. Nominal visits will be used for the by-visit summaries, with the worst/highest toxicity grade value presented for the visit in the case of multiple reported observations.

4.2 Primary Estimand Analysis

4.2.1 Definition of Endpoint

SARS-CoV-2 neutralizing antibody titers (Wuhan strain, presented in WHO standardized units of IU/mL) at 2 weeks after the last vaccination, i.e., after the second vaccination in initially seronegative subjects (Group 1) and after the single boost vaccination in initially seropositive subjects (Groups 2 and 3), for subjects in the Immunogenicity Analysis Set (Section 4.1.2). For Group 1, this will be the neutralizing antibody titer collected at Week 6, and for Groups 2 and 3 it will be the neutralizing antibody titer collected at Week 2.

Serum samples will be collected from subjects as outlined in the trial procedure schedule Appendix 2 and Appendix 3. Immunogenicity testing will be performed at [redacted] and at contracted laboratories if needed.

Neutralizing antibody titers to SARS-CoV-2 are assumed to be log-normally distributed. Values will be transformed to the $\log_{10}$ scale for the purpose of analysis. Imputation of values based on limits of detection and quantitation are described in Section 4.1. Once analyses are performed, estimates will be back transformed to the original scale for reporting.

The intercurrent event that may have an impact on the primary estimand is COVID-19 infection prior to the Week 6 or Week 2 neutralizing antibody titer collection, respectively, for all groups. As subjects who come in contact with the virus can have significant interference with the SARS-CoV-2 neutralizing antibody levels, these subjects will be excluded from the primary analysis. This is equivalent to the "while on treatment" strategy. Reasons for exclusion from the primary estimand analysis will be summarized separately for full accounting of the study population.

The second intercurrent event of discontinuing the study prior to collection of the neutralizing antibody titer endpoint at Week 6 or Week 2, respectively, for each group is not expected to have an impact on SARS-CoV-2 neutralizing antibody levels. Therefore, the "treatment policy" strategy will be used in these cases.

As the primary estimand is specifically defined for subjects who meet the criteria to be in the Immunogenicity Analysis Set, subjects who have protocol deviations that would substantially affect the neutralizing antibody titer levels at the respective primary analysis timepoints are not included in the analysis. These subjects will be summarized in the disposition displays as being excluded from the Immunogenicity Analysis Set, as well as summarized in a table as to which deviations were observed that may have had a substantial effect on their primary endpoint value.

4.2.2 Main Analytical Approach

The estimate of the primary estimand will be created using the Immunogenicity Analysis Set, which will include only subjects who have no protocol deviations that will substantially affect the immunogenicity outcomes. Due to this, missing values for the primary estimand analysis are unexpected. Subjects who have a value for the primary time point but developed COVID-19 symptoms prior to 2 weeks after the last vaccination, and had a positive PCR result for SARS-CoV-2 infection prior to the 2 weeks after the last vaccination time point, will be excluded from the analysis due to the interference the infection will have on the resulting neutralizing antibody results.

The geometric mean of SARS-CoV-2 neutralizing antibody titers (Group 1), and fold increases (Groups 2 and 3) 2 weeks after the last vaccination, will be summarized along with their 95% CIs. No statistical hypothesis testing will be performed for this single arm trial.

Geometric mean titers (GMTs) will be obtained by computing the arithmetic means and the corresponding 95% CIs on the $\log_{10}$ scale, and then exponentiating the $\log_{10}$ means and confidence limits to return the results to the original scale.

Fold increases for Groups 2 and 3 will be calculated as the ratio of the SARS-CoV-2 neutralizing antibody titer at 2 weeks post-last vaccination to the baseline neutralizing antibody titer (i.e., last measurement prior to vaccination). Geometric mean fold increases (GMFIs) will be calculated in the same manner as GMTs.

4.2.3 Sensitivity Analyses

The reason for exclusion from the primary estimand analysis will be included with the sensitivity analysis for a full account of the study population. Reasons may include, but are not limited to:

- Development of COVID-19 symptoms and having a positive PCR result for SARS-CoV-2 infection prior to 2 weeks after the last vaccination
- A protocol deviation substantially affecting the immunogenicity outcome (i.e., exclusion from the Immunogenicity Analysis Set)

4.2.3.1 Sensitivity using the Safety Analysis Set

The analytical approach used for the primary estimand will be repeated using the Safety Analysis Set including any available post-last vaccination value for the primary time point (2 weeks post-last vaccination) regardless of window, and without respect to protocol deviations that may substantially affect immunogenicity outcomes. If multiple post-last vaccination neutralizing titer values are available, the value closest to the target day (2 weeks post last vaccination, i.e., Week 6 for Group 1 and Week 2 for Group 2 and Group 3) will be used for the sensitivity analysis. If two values are equidistant from the target day, the later value will be used. No pre-vaccination titer values will be analyzed as post vaccination titer results.

4.2.3.2 Sensitivity using Multiple Imputation for the Safety Analysis Set

Because the window between vaccination and the primary endpoint measurement is short, the number of subjects with missing data due to intercurrent events or protocol deviations substantially affecting the immunogenicity results is expected to be small. If data are missing despite the short window, missing values will be imputed via the multiple imputation (MI) method as a sensitivity analysis using the Safety Analysis Set. Missing values will be assumed to be missing at random, such that the reason for the missing value is not dependent on the result itself. As the MI procedure will be performed on the Safety Analysis Set, regardless of protocol deviations, post vaccination neutralizing titers that are out of window will be included rather than imputing a "missing" value for full accounting of the available data. Inclusion of out of window titer results will follow the method used for the Sensitivity Analysis using the Safety Analysis Set.

The multiple imputation sensitivity analyses will be performed separately for each group due to the different background characteristic of the two populations. Although analyses for Groups 2 and 3 are based on the fold-increase from baseline neutralizing titers, imputation of missing data will be performed at the neutralizing titer level, with fold-increase computed after imputation.

Group 1 Multiple Imputation Procedure

For Group 1, assuming the post-vaccination $\log_{10}$ titer values are normally distributed, MI will be used to create 100 complete data sets that will account for the random variability in the $\log_{10}$ neutralizing titer values. Week 2, Week 4, and Week 5 $\log_{10}$ neutralizing titer results, number of vaccinations received, year of birth, sex, and race will be used in the joint model to predict the $\log_{10}$ neutralizing titer value for the primary estimand time point at Week 6.

Qualitative tests for SARS-CoV-2 antibodies are required for inclusion in the trial and appropriate placement of subjects into their respective seronegative or seropositive groups. As Group 1 subjects are required to be seronegative at screening, most Baseline neutralizing titers are expected to be below the LLOQ. As this would provide little information to the joint distribution from which to draw the MI samples, Baseline neutralizing titer values will not be included in the Group 1 MI procedure.

In addition, as the missing pattern of titer values may not be monotone, i.e., subjects may be missing Week 2, Week 4, Week 5, or Week 6 neutralizing titer results, or a combination of these values, a fully conditional specification (FCS) method will be used. This method assumes the existence of a joint distribution for all variables used to predict the missing values and allows Week 2, Week 4, and Week 5 $\log_{10}$ titer value to be imputed, if needed, to inform the missing Week 6 neutralizing titer values.

MI analyses will be performed in SAS using the PROC MI and PROC MIANALYZE procedures. A minimum of $\log_{10}$ of half of the LLOQ will be set for imputed values to correspond to the observed data range. In addition, a seed of 19201 will be used for the procedure. The following is the sample code for the MI sensitivity analyses of the primary estimand for Group 1.

titersl: the dataset containing the $\log_{10}$ neutralizing titer data along with the covariates used in the analysis, one record per subject.
sex: The sex identifier
yob: Year of birth
race: The race identifier
nvacc: Number of vaccinations received (1 vs. 2)
ltiterW2: Log_{10} Week 2 Titer Result
ltiterW4: Log_{10} Week 4 Titer Result
ltiterW5: Log_{10} Week 5 Titer Result
ltiterW6: Log_{10} Week 6 Titer Result

```
proc mi data=titersl out=titermi nimpute=100 seed=19201 minimum=[log10LLOQ/2];
    class nvacc sex race;
    var  nvacc yob sex race ltiterW2 ltiterW4 ltiterW5;
    fcs nbiter=20 reg(ltiterW6 = ltiterW2 ltiterW4 ltiterW5 nvacc yob sex race
                      /details);
run;

proc means data=titermi noprint nway;
 by _imputation_;
 var  ltiterW6;
 output out=summ(drop=_type_ _freq_) n=n mean=logmn stderr=logse;
run;

ods output parameterestimates=group1est;
proc mianalyze data=summ;
    modeleffects logmn;
    stderr logse;
run;
data group1est2;
    set group1est;
    GMT=10**estimate;
    GMT_LCL=10**LCLmean;
    GMT_UCL=10**UCLMean;
    keep estimate lclmean uclmean gmt:;
    label estimate='Mean Log10 Titers'
          lclmean='Lower 95% CI of the Mean Log10 Titers'
          uclmean='Upper 95% CI of the Mean Log10 Titers'
          gmt='Geometric Mean Titer'
          GMT_LCL='Lower 95% CI of the Geometric Mean Titer'
          GMT_UCL='Upper 95% CI of the Geometric Mean Titer'
run;
```

Groups 2 and 3 Multiple Imputation Procedure

For Groups 2 and 3, again assuming the $\log_{10}$ titer values are normally distributed, MI will be used to create 100 complete data sets that will account for the random variability in the neutralizing titer values. Group 2 and 3 analyses will be performed separately, but using the same method. Baseline and Week 1 $\log_{10}$ neutralizing titer values, stratification subgroup, year of birth, sex, and race will be used in the joint model to predict the $\log_{10}$ neutralizing titer value for the primary estimand time point at Week 2. Qualitative tests for SARS-CoV-2 antibodies are required for inclusion in the trial and appropriate placement of subjects into their respective seronegative or seropositive groups. However, the chance for missing Baseline neutralizing titer values still exists. In addition, as the missing pattern of titer values may not be monotone, i.e., subjects may be missing Baseline neutralizing titer values, Week 1, or Week 2 neutralizing titer values, or a combination of these values, a FCS model will be used, assuming the existence of a joint distribution for all variables used to predict the missing values and allowing the Baseline and Week 1 $\log_{10}$ titer results to be imputed, if needed, to inform the missing Week 2 neutralizing $\log_{10}$ titer result.

For missing Baseline and Week 1 neutralizing titer values in Groups 2 and 3, titer results can still be assumed log-normally distributed. Using the FCS regression model, missing neutralization titer results will be imputed using the simulated from the posterior predictive distribution of the parameters Baseline and Week 1 log_{10} neutralizing titers, stratification subgroup, year of birth, sex, and race.

After the creation of the 100 complete datasets, the fold-increase for Week 2 versus Baseline neutralizing titer results, i.e., the difference in the values on the log_{10} scale, will be created for all Safety Analysis Set subjects. The mean difference in log_{10} titers from Baseline to Week 2 and corresponding standard errors will be calculated within each imputation, and combined over the 100 imputations. The combined results will be presented on the log_{10} scale using means and 95% CIs, as well as back transformed to present the geometric mean fold-increases and associated 95% CIs on the original scale.

As with Group 1, MI analyses will be performed in SAS using the PROC MI and PROC MIANALYZE procedures. A minimum of log_{10} of half of the LLOQ will be set for imputed values to correspond to the observed data range. In addition, a seed of 19201 will be used for the procedure. The following is the sample code for the MI sensitivity analyses of the primary estimand for Groups 2 and 3.

titersl: the dataset containing the log_{10} neutralizing titer data along with the covariates used in the analysis, one record per subject.
sex: The sex identifier
yob: Year of birth
race: The race identifier
stratgoup: Stratification subgroup (prior mRNA vaccine, prior Adenovirus-based vaccine, prior COVID-19 infection)
ltiterBL: Log_{10} Baseline Titer Result
ltiterW1: Log_{10} Week 1 Titer Result
ltiterW2: Log_{10} Week 2 Titer Result
lfoldinc: Log_{10} Fold Increase from Baseline to Week 2 Titer Result

```
proc mi data=titersl out=titermi nimpute=100 seed=19201 minimum=[LLOQ/2];
    class stratgroup sex race;
    var  stratgroup yob sex race ltiterBL ltiterW1;
    fcs nbiter=20 reg(ltiterW2 = ltiterBL ltiterW1 stratgroup yob sex race
                                /details);
run;

data titersmi2;
      set titersmi;
      lfoldinc= ltiterW2 - ltiterBL;
run;

proc means data=titermi2 noprint nway;
```

```
 by _imputation_;
 var  lfoldinc;
 output out=summ(drop=_type_ _freq_) n=n mean=logmn stderr=logse;
run;

ods output parameterestimates=group2est;
proc mianalyze data=summ;
    modeleffects logmn;
    stderr logse;
run;
data group2est2;
    set group2est;
    GMFI=10**estimate;
    GMFI_LCL=10**LCLmean;
    GMFI_UCL=10**UCLMean;
    keep estimate lclmean uclmean GMFI:;
    label estimate='Mean Log10 Fold Increase'
          lclmean='Lower 95% CI of the Mean Log10 Fold Increase'
          uclmean='Upper 95% CI of the Mean Log10 Fold Increase
          GMFI='Geometric Mean Fold Increase'
          GMFI_LCL='Lower 95% CI of the Geometric Mean Fold Increase'
          GMFI_UCL='Upper 95% CI of the Geometric Mean Fold Increase'
          ;
run;
```

4.2.4 Supplementary Analyses

The primary analysis will be supported by descriptive summaries including medians, geometric means and corresponding 95% CIs, Min, and Max for each serum collection time point. These summaries will be performed for both the Immunogenicity Analysis Set and the Safety Analysis Set. No windowing or imputation will be applied for this summary, as they will be presented by the visit at which the serum sample was acquired.

A responder analysis will be performed, with response analyzed by serum collection time point. Response defined separately for naïve subjects (Group 1) and experienced subjects (Groups 2 and 3) as follows:

- Group 1: having SARS-CoV-2 neutralizing antibody titers above the LLOQ, or
- Groups 2 and 3: having an at least 2-fold, 4-fold, and/or 6-fold increase from baseline SARS-CoV-2 neutralizing antibodies.

For Group 1, subjects are required to have a negative qualitative SARS-CoV-2 test at screening to enter the trial. Despite this, there is a chance they have positive neutralizing titer at baseline. In this case, response would be defined as an at least 2-fold increase from baseline in neutralizing titer.

Response rates will be summarized for the Immunogenicity Analysis Set by time point as well as overall for the Active Trial Period, and corresponding 95% CIs will be calculated using the Clopper-Pearson method. The response rate analysis will also be repeated for the Safety Analysis Set with subjects missing a value for a time point, and not having achieved a response at a previous timepoint, counted as non-responders. For the final CSR, an over response rate will be calculated inclusive of the FU visits in addition to the Active Trial Period rates.

The primary analyses will also be performed within sex (male vs. female) and age groups as supportive analyses: 18 to < 50 years, 50 to < 65 years, 65 to < 75 years, and ≥ 75 years. These summaries will be performed for the Immunogenicity Analysis Set only.

4.3 Secondary Estimand Analyses

4.3.1.1 Definition of Endpoints

Percent of subjects reporting any SAE or AESI, as well as ≥ Grade 3 AEs assessed as related to trial vaccine within 8 days after vaccination.

The second intercurrent event, discontinuation due to AEs from either the second vaccination (Group 1) or the trial (all groups), is relevant for this estimand. If it is confirmed by the investigator that the subject discontinues the trial within 8 days after vaccination due to AEs, the subject is considered having a related SAE/AESI/Grade 3 or higher AE regardless of the seriousness, severity, or causality of the AE for which the subject discontinued. This is consistent with the "composite" strategy.

4.3.1.2 Main Analytical Approach

For the Safety Analysis Set, the percent of subjects reporting any related SAE or AESI, as well as subjects reporting any related ≥ Grade 3 AE within 8 days after any vaccination will be summarized, and the 95% CI will be calculated using the Clopper-Pearson method. For Group 1, this analysis will be performed by vaccination period and for the overall Active Trial Period.

4.3.1.3 Sensitivity Analyses

If any subject discontinues the trial within 8 days after vaccination due to AEs, a sensitivity analysis will be performed using the "treatment policy" strategy to handle the intercurrent event, where the subject will not be considered having a related SAE or AESI, or having a ≥ Grade 3 AE assessed as related to trial vaccine, unless the specified AE leading to discontinuation meets one or more of these categories.

4.3.1.4 Supplementary Analyses

The secondary estimand analysis will be repeated for the percent of subjects reporting any related SAE or AESI, as well as subjects reporting any related ≥ Grade 3 AE within 29 days after any vaccination as a supplementary endpoint.

Additional analyses of AEs will be included as part of the safety analyses.

4.4 Exploratory Endpoints Analyses

Exploratory endpoints will be performed using the Immunogenicity Analysis Set with available data for the endpoint of interest. Tables will be structured similarly to the primary estimand analysis with Groups 2 and 3 having summaries by baseline Wuhan strain neutralizing antibody titer.

SARS-CoV-2 neutralizing antibody titers against variant strains circulating at the time of analysis (e.g., alpha, beta, delta, and omicron strains) at all available time points will be summarized descriptively using geometric means and 95% CIs for each time point and group.

Total antibody titers measured by ELISA and cellular immune responses measured by ELISPOT will also be summarized using geometric means and their 95% CIs similar to the summaries performed for the neutralizing antibody titers. ELISPOT results will be presented by stimulant pool for Interferon-γ and Interleukin-4.

The geometric means and 95% CIs, as well as fold increases, for both neutralizing antibody titers and total antibody titers will be plotted as a function of time within group, respectively. Response rates as described in Section 4.2.4 will also be plotted by time point, variant strain, and group.

Pearson's correlation coefficient and 95% CI between the $\log_{10}$-transformed neutralizing and total antibody titers 2 weeks after the last vaccination will be calculated within group along with the associated p-value. These will also be presented in scatter plots with neutralizing antibodies on the x axis and total antibodies on the y axis.

4.5 Other Safety Analyses

Safety data will be summarized descriptively using the Safety Analysis Set. AE and medical history data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 24.0, and reported using System Organ Classes (SOCs) and Preferred Terms (PTs). Prior and concomitant medications will be coded to World Health Organization (WHO) Drug Dictionary version March 2021, and will be reported by Anatomic-Therapeutic-Chemical (ATC) Class Level 2 and Preferred Name.

4.5.1 Extent of Exposure

Exposure to trial vaccine will be summarized by group, including the number of vaccinations received as well as the number and percentage of subjects returning memory aids for the Safety Analysis Set. All exposure data will be listed.

4.5.2 Adverse Events

AEs are defined as any untoward medical occurring after a subject has signed informed consent form, and does not necessarily have a causal relationship associated with the administration of the trial vaccine.

An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- Is an otherwise important medical event

The criteria for an event being serious will be captured in the AE case report form as reported by the investigator.

AESIs are defined per the Safety Platform for Emergency vACcines (SPEAC) Project generated list of AESI for safety monitoring, and will be flagged as such in the AE case report form by the investigator. For analyses, the investigator assessment as recorded on the AE case report form will be used to identify these AESIs.

4.5.2.1 Solicited Adverse Events

Solicited AEs are defined as all symptoms specifically listed in the memory aid provided to the subjects following each vaccination. After vaccination, the subjects are requested to monitor and record local symptoms (i.e., erythema, swelling, induration, pruritus and pain at the injection site) as well as general symptoms (i.e., body temperature increase/pyrexia, headache, chills, myalgia, nausea and fatigue) in the memory aid daily for the day of vaccination and the following 7 days (Days 1 to 8 following vaccination, 8-day period). If symptoms persist at Day 8, daily symptoms and temperatures will be documented each day until resolved. Solicited AEs that meet the criteria for SAEs will be documented in the AE case report form as well as the memory aids.

As Group 1 receives 2 vaccinations, summaries will be presented by vaccination period and for the overall Active Trial Period. For Groups 2 and 3, there will be a single summary for the Active Trial Period.

Solicited Local AEs

Occurrence, intensity, and duration of solicited local AEs during the 8-day period after vaccination will be summarized for each group. Note, solicited local AEs are always assumed to be related to vaccination so no summaries based on causality will be performed.

Injection site erythema, swelling and induration will be measured using a provided ruler and the maximum diameter will be recorded for each day on the memory aid. The intensity for these symptoms will be graded as follows:

Table 3 Solicited Local Event Grading

MedDRA coded Preferred Term	Grade	Intensity
Injection site erythema, swelling, and induration	0	0
	1	< 30 mm
	2	≥ 30 – < 100 mm
	3	≥ 100 mm
Injection site pruritus	0	Absent
	1	Mild
	2	Moderate
	3	Severe
Injection site pain	0	Absent
	1	Painful on touch
	2	Painful when limb is moved
	3	Spontaneously painful/prevents normal activity

Solicited General AEs

Occurrence, relationship, intensity, and duration of solicited general AEs during the 8-day period after each vaccination will be summarized for each group based on the grades defined below:

Table 4 Solicited General Event Grading

MedDRA coded Preferred Term	Grade	Intensity
Body temperature*	0	< 37.5°C (< 99.5°F)
	1	≥ 37.5 – < 38.0°C (≥99.5 – <100.4°F)
	2	≥ 38.0 – < 39.0°C (≥100.4 – <102.2°F)
	3	≥ 39.0 – < 40.0°C (≥102.2 – <104.0°F)
	4	≥ 40.0°C (≥ 104.0°F)
Headache, Myalgia, Nausea, Chills and Fatigue	0	None
	1	Mild: easily tolerated, minimal discomfort and no interference with daily activity
	2	Moderate: Some interference with daily activity
	3	Severe: Prevents daily activity

*Pyrexia is defined as oral temperature ≥ 38.0^{0}C (≥ 100.4^{0}F).

Causal relationship between solicited general AEs and the vaccine will be assessed by the investigator using the same categories as for unsolicited AEs (see Clinical Trial Protocol Section 8.1.8).

Analysis

Solicited AEs will be summarized by AE term for overall occurrence and by maximum intensity using frequencies and percentages. Note, only solicited events with intensity grades above 0 are considered AEs, however subjects reporting intensity grades of 0 for the collection period will be included in summaries to account for all observed memory aid data. As intensity is still collected daily for ongoing events after day 8 of the memory aid reporting period, the maximum intensity used in summaries will be derived from the entire duration of the event and not just from the 8-day reporting period.

Group 1 summaries will be performed by period (Vaccination Period 1, Vaccination Period 2, and for the overall Active Trial Period). Groups 2 and 3 will be summarized for the Active Trial Period, separately from Group 1.

In addition, duration of solicited events will be summarized only for subjects experiencing the event of interest using n, mean, SD, median, Min, and Max. Although the collection period is 8 days including the day of vaccination, durations may be longer than 8 days if the solicited event is ongoing at Day 8. For subjects in Group 1 who receive two vaccinations, the maximum duration across the two vaccination periods will be reported for the Active Trial Period summary.

Additional summaries of the subset of general AEs determined to be related to vaccination, as well as those considered both related and ≥ Grade 3, will also be presented using frequencies and percentages.

The solicited AE information from the memory aid as well as the physician's assessments and durations will be included in subject-level listings.

4.5.2.2 Unsolicited Adverse Events

All intercurrent diseases reported when the investigator actively inquires the subject will be documented as unsolicited adverse events. Unsolicited AEs will be assessed and documented from ICF signature through EAP, and if ongoing at that time followed until resolution or until the subject's last trial visit, at the latest. SAEs and AESIs will be collected through the end of the trial, including during the FU period, and followed-up until resolution or achievement of stable clinical conditions.

Relationship of the trial vaccine to the AE is assessed by the investigator using the categories "none," "unlikely," "possible," "probable," and "definite." An AE assessed as possibly, probably, or definitely related will be counted as a related AE for the purpose of reporting. AEs categorized as none or unlikely for causality will not be considered related to trial vaccination. AEs missing a relationship will not have a causality imputed.

Unsolicited AEs are graded based on the *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* (FDA, 2007). The maximum toxicity grade over the duration of the AE will be reported in the case report form. If a subject has multiple AEs within the same PT, the highest toxicity grade will be used for subject-level summaries. Grading will be based on the descriptions listed in Table 5.

Table 5 Adverse Event Grading

Grade	Definition
Grade 1	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with daily activities.
Grade 2	An AE which is sufficiently discomforting to interfere with daily activities, but does not require medical intervention (non-narcotic pain reliever or other nonprescription medication are not considered "medical intervention" for this purpose).
Grade 3	An AE which prevents daily activities and which requires medical intervention (non-narcotic pain reliever or other nonprescription medication are not considered "medical intervention" for this purpose)
Grade 4	Life-threatening, or disabling.

Analysis

The secondary estimand analyses for SAEs, AESIs, and related ≥ Grade 3 AEs within 8 days after vaccination are described in Section 4.3.

In general, AEs will be summarized by SOC, PT, and group for the Safety Analysis Set in the Active Trial Period. AEs occurring after the Active Trial Period during the FU period will be included in the AE listings. AEs occurring between ICF signature and first vaccination will be

considered Baseline Signs and Symptoms and will be reported similarly to medical history events.

For Group 1, summaries will also be included by period for Vaccination Period 1, Vaccination Period 2, and the overall Active Trial Period. For the overall Active Trial Period, event counts will include events from both vaccination periods in the case a subject experiences the same event multiple times.

Groups 2 and 3 will be summarized for the Active Trial Period, separately from Group 1.

An overall summary of solicited and unsolicited AEs including subject counts, event counts, percentages (of subjects only) will be created for the following event categories:

Solicited and Unsolicited AEs

- All AEs
- SAEs
- AEs Leading to Withdrawal
- Fatal AEs

Solicited AEs

- Local AEs
  - ≥ Grade 3
  - Leading to Deferral or Discontinuation of Vaccine
  - SAEs
- General AEs
  - ≥ Grade 3
  - Related
  - Related ≥ Grade 3
  - Leading to Deferral or Discontinuation of Vaccine
  - SAEs

Unsolicited AEs

- All AEs
  - Related
  - ≥ Grade 3
  - Related ≥ Grade 3

  - Leading to Withdrawal
- SAEs
  - Within 8 Days After Vaccination
  - Within 29 Days after Vaccination*
  - Related SAEs
  - Fatal
- AESIs
  - Within 8 Days After Vaccination
  - Within 29 Days after Vaccination*
  - Related AESIs
- Related AEs ≥ Grade 3
  - Within 8 Days After Vaccination
  - Within 29 Days after Vaccination*

*Within 29 days summaries includes through the day prior to second vaccination for Group 1 Vaccination Period 1, or from the last/single booster vaccination through the end of the Active Trial Period for all groups. The window for the end of the Active Trial Period visit is 28-35 days post last/single booster vaccination.

Summary tables for unsolicited AEs by SOC and PT will be presented including subject and event counts and percentages (of subjects only) for the Active Trial Period:

- AEs
- Related AEs
- AEs ≥ Grade 3
- Related AEs ≥ Grade 3
  - Related AEs ≥ Grade 3 Occurring within 8 Days of any Vaccination
  - Related AEs ≥ Grade 3 Occurring within 29 Days of any Vaccination*
- Non-serious AEs in > 5% of Subjects
- SAEs
- Related SAEs
  - Related SAEs Occurring within 8 Days of any Vaccination
  - Related SAEs Occurring within 29 Days of any Vaccination*
- AESIs

- Related AESIs
  - Related AESIs Occurring within 8 Days of any Vaccination
  - Related AESIs Occurring within 29 Days of any Vaccination*
- AEs Leading to Discontinuation of Active Trial Period

*Within 29 days summaries includes through the day prior to second vaccination for Group 1 Vaccination Period 1, or from the last/single booster vaccination through the end of the Active Trial Period for all groups. The window for the end of the Active Trial Period visit is 28-35 days post last/single booster vaccination.

Tables summarized by SOC and PT will be sorted in order of descending incidence of SOCs in the overall column, and descending order of incidence of PTs within the SOCs. For subject level frequencies and percentages, subjects experiencing an event more than once will be counted only once within SOC and PT, however all events will be counted in the event column.

All AEs will be listed by group, subject, onset date, SOC, and PT. Separate listings will be created for SAEs, AESIs, AEs ≥Grade 3, and AEs leading to discontinuation of the Active Trial Period. Listings will include any AEs collected during the FU period.

4.5.3 Additional Safety Assessments

4.5.3.1 Electrocardiograms

ECGs are only required to be performed at screening to confirm the subject is eligible for the trial. Post-vaccination ECGs are performed only if clinically indicated; therefore, results from ECGs will be included only in subject level listings.

4.5.3.2 Vital Signs

Vital signs will be graded as in Table 6 below, per the *Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials* (FDA 2007).

Table 6 Vital Signs Grading

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

ER = emergency room; mm Hg = millimeters of mercury.

Note, the Grade 4 categories requiring a corresponding emergency room visit or hospitalization will not be included in this analysis, as they should be reported separately as SAEs. The frequency and percentage of subjects experiencing Fever, Tachycardia, Bradycardia, Hypertension (systolic and diastolic), and Hypotension (systolic) will be summarized for the Active Trial Period for any occurrence and by grade within vaccination group.

4.5.3.3 Laboratory Results

A list of laboratory parameters collected is included in Section 8.1.10 of the Clinical Trial Protocol. For the purpose of analysis, laboratory data will be converted to standard Système International d'Unités (SI) units during creation of the Study Data Tabulation Model (SDTM) datasets. The original laboratory values and units will also be stored in the SDTM datasets. Only the SI units will be used in tables and listings. SI units and conversions will be included in the SDTM documentation.

All measured laboratory values will be listed and summarized at each scheduled visit using descriptive statistics. Out of range laboratory values will be flagged as either "L" for below normal range or "H" for above normal range in listings. Clinically significant abnormal laboratory values are recorded as AEs and summarized along with the unsolicited AEs.

Toxicity will be graded based on the *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* (FDA 2007). Grade 1 or Grade 2 toxicity is only graded according to this scale if the value is outside of the institutional normal range applicable for this trial.

Shift tables will be used to evaluate categorical changes in toxicity levels from baseline to each schedule time point, as well as to worst toxicity grade during the Active Trial Period, for laboratory parameters graded per the above toxicity scale.

For laboratory parameters not graded per the above toxicity scale, similar shift tables will be created based on the laboratory provided normal ranges (Low, Normal, High). Summary tables will be produced for the number of high and low laboratory values at each scheduled time point by laboratory category and parameter.

Any laboratory parameters which are not included in the protocol but reported by the laboratories (e.g., in order to define an AE) will be listed but not tabulated. Pregnancy test results will also be included only in listings.

4.5.3.4 SARS-CoV-2 Infection Results via PCR

The frequency and percentage of subjects testing positive for COVID-19 infection by visit, any infection prior to 2 weeks post-last vaccination, any infection after the 2 weeks post-last vaccination time point, and any infection post vaccination overall will be summarized by group. As collection may occur during unscheduled visits, frequencies and percentages will include all subjects who tested positive between the prior visit through the summarized visit.

4.5.3.5 Physical Examinations

The performance of physical examinations will be listed. Findings upon physical examination will be added to the Medical History case report form if they started prior to the signing of the ICF, and to the AE case report form page if starting afterward. Findings occurring after the signing of the ICF, but pre-vaccination will be categorized as baseline signs and symptoms, and post-vaccination events through the end of the Active Trial Period visit will be considered part of the Active Trial Period. Findings occurring after the Active Trial Period will be considered FU period AEs and only included in AE listings.

4.5.4 Subgroup Analyses

No additional subgroup analyses will be performed for safety endpoints.

4.6 Interim Analyses

Description of the run-in phase of the trial is included in Section 1.2. In order to inform the phase 3 trial, a primary analysis of immunogenicity and safety will be performed once all Group 2 and 3 subjects have completed the end of the active phase visit or withdrawn early from the trial, and at least the neutralizing antibody testing results supporting the primary endpoint are available. Trial data will be cleaned through the end of the Active Trial Period visit for these subjects, and a data review meeting will be conducted to review the key trial data for the primary analysis and determine the subjects to be excluded from the Immunogenicity Analysis Set.

The primary analysis will include both the primary and secondary estimand analyses, as well as the available safety, demographics, and disposition data through the end of the Active Trial Period for Groups 2 and 3. In addition, the total antibody data and neutralizing antibody titers against various variant strains at 2 weeks after last vaccination will be provided, if available.

The results of the primary analyses from the primary and secondary estimand analyses are not expected to change for later reporting, nor is statistical hypothesis testing to be performed. Therefore, the analyses for the primary estimand for Groups 2 and 3 performed at this time will be considered “final” for the purposes of this trial and no adjustments for multiple reviews of the data are required.

The final analysis for the trial will occur once all subjects have completed the follow up visits or withdrawn early from the trial and the database has been locked. Group 1, follow-up, and safety data through the end of the trial, as well as additional immunogenicity and SARS-CoV-2 infection testing performed at 3 months for all groups, as well as 6 months, 1 year, and 2 years for Groups 2 and 3 subjects will be reported in this analysis as an appendix to the primary analysis. These data will be in addition to the already reported trial results rather than a “re-analysis” of already reported endpoints. Although unlikely, any changes in the data or planned analyses from the primary analysis will be fully documented.

4.7 Changes to Protocol-planned Analyses

The primary analysis has been updated to include only Groups 2 and 3 through the end of the Active Trial Period to assist in planning for the Phase 3 trial.

For neutralizing titer response in seropositive subjects, a threshold of ≥ 6-fold increase has been added to the response summaries.

5 Subject Population Summaries

5.1.1.1 Disposition

All subjects screened will be accounted for in disposition summaries. A summary table will be presented specifying the number of subjects screened, vaccinated, completing the Active Trial Period, included in each analysis set, withdrawing prior to the second vaccination (Group 1 only, and reason), withdrawing during the Active Trial Period (and reason), and completing each FU visit.

A listing will present all vaccinated subjects, date of trial completion/discontinuation, date(s) of vaccination(s), and reason for withdrawal from Active Trial Period or early discontinuation from the trial as a whole. All subjects who signed ICF but are not eligible for the trial will be listed, including the reason for ineligibility.

5.1.1.2 Analysis Populations

Frequencies and percentages of each analysis population will be presented by group and overall. Reasons for exclusion from the Immunogenicity Analysis Set will be included in the subject-level listings.

5.1.1.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol. The noncompliance may be either on the part of the subject, the investigator, or the trial site staff. Protocol deviations are collected on both a site and subject level basis. Subject level deviations will be databased and listed. Categorized deviations will be presented using frequencies and percentages for the Safety Analysis Set.

5.1.1.4 Demographics and Baseline Characteristics

Demographic Variables

- Age at Informed Consent [years]
- Age Group (18 to < 50 years, 50 to < 65 years, 65 to < 75 years, and ≥ 75 years)
- Sex (Male, Female)
- Race (White, Black or African American, Asian, Native Hawaiian or Other Pacific Islander, American Indian or Alaska Native, Multiple, Not Reported)
- Race Group (White, Other)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino)
- Height [cm]

- Body weight [kg]
- BMI [kg/m^2]
- Reason for Seropositivity (Groups 2 and 3)
- Primary Vaccine Received (Groups 2 and 3)
- Baseline Wuhan Strain Neutralizing Titer Value Category
  - <LLOD
  - LLOD - <LLOQ
  - ≥LLOQ
  - LLOQ - <Median (median of titers at or above LLOQ in Groups 2 and 3)
  - ≥Median

Analyses

Listings will be presented for all data in the database. Tables of descriptive statistics for demographics will be produced for the Safety Analysis Set and the Immunogenicity Analysis Set. For Groups 2 and 3, summaries will also be presented by baseline Wuhan Strain Neutralizing Titer Value Category. Descriptive statistics will be presented for the continuous demographic variables. Categorical demographic and baseline variables will be summarized using frequencies and percentages.

5.1.1.5 Medical History and Baseline Signs and Symptoms

Medical history data are collected at screening and include conditions that started prior to signing of the ICF. These data are coded to MedDRA dictionary SOCs and PTs. Summaries of medical history events will be created by SOC and PT for the Safety Analysis Set. SOCs and PTs within SOCs will be sorted by descending frequency in the overall column.

Baseline Signs and Symptoms are defined as AEs that occur between the signing of informed consent and the first vaccination. These events are reported along with the AEs in the case report form, but will be summarized separately from AEs similar to the medical history population summary. Like medical history events, they will be coded to MedDRA SOCs and PTs. Baseline Signs and Symptoms will be listed by SOC and PT within each subject.

5.1.1.6 Prior and Concomitant Medication

Displays of **prior** medications include medications where end date is before date of first administration of trial vaccine. Displays of **concomitant** medications include all ongoing medications, medications with missing end dates, or medications with end date after the first administration of trial vaccination. Tables by ATC Level 2 class and preferred name will be

presented for the Safety Analysis Spet. If ATC Level 2 class is not available, the next highest class available will be used. Subject level listings will be created by group, subject, ATC Level 2 class, preferred name, and verbatim term.

6 Sample Size Determination

Group 1: approximately 30 subjects determined to be seronegative for SARS-CoV-2 antibodies at screening will be enrolled in Group 1, 100 µg dose.

Group 2: approximately 90 subjects determined to be seropositive for SARS-CoV-2 antibodies at screening will be enrolled in Group 2, 100 µg dose.

Group 3: approximately 90 subjects determined to be seropositive for SARS-CoV-2 antibodies at screening will be enrolled in Group 3, 50 µg dose.

The primary objective of Group 1 is to estimate the level of neutralizing antibodies 2 weeks after the last (i.e., second) vaccination. This will be measured by the geometric mean of the neutralizing antibody titers and its 95% CI. Based on published data on the peak neutralizing antibody titers following vaccination with licensed SARS-CoV-2 vaccines, we assume the SD in logarithm 10 ($\log_{10}$) scale will be approximately 0.5. When the sample size is 30, a two-sided 95% CI will extend 0.179 from the observed mean in $\log_{10}$ scale. The corresponding 95% CI for the geometric mean will be between 132 and 302 if the point estimate is 200; it will be between 166 and 377 if the point estimate is 250. The lower bound of 132 to 166 is in the similar range of licensed SARS-CoV-2 vaccines (Jin et al., 2021).

The primary objective of Groups 2 and 3 is to estimate the geometric mean of the ratio of neutralizing antibody titers 2 weeks after the single booster vaccination with ABNCoV2 versus the pre-booster level. We assume the same SD of 0.5 in $\log_{10}$ scale in both pre-booster and post-booster titers; the correlation coefficient between the pre-booster and post-booster titers is 0.3; and the distribution of the ratio is log normal. When the sample size is 90, a two-sided 95% CI will extend 0.122 from the observed mean in $\log_{10}$ difference. If the observed geometric mean of the ratio is 4 (i.e., a 4-fold increase after booster), the 95% CI will be (3.0, 5.3). When stratified by baseline titer subgroups (< median or ≥ median), the lower bound of the 95% confidence interval will be at least 2.7 when the observed ratio is 4 with a subgroup size of 45.

Appendix 1: List of Abbreviation and Term Definitions

Abbreviation	Definition
ABNCoV2	SARS-CoV-2 trial vaccine
AE	adverse event
AESI	adverse event of special interest
ATC	Anatomic-Therapeutic-Chemical Class
Active Trial Period	The period from the first vaccination up to and including 1 month (28-35 days) after receiving the last vaccination.
BN	Bavarian Nordic
BMI	body mass index
CI	confidence interval
CSR	clinical study report
CTS	clinical trial site
COVID-19	coronavirus disease 2019
DMC	data monitoring committee
ECG	electrocardiogram
EDC	electronic data capture
EAP	End of Active Trial Period
ELISPOT	enzyme-linked ImmunoSpot technique
ELISA	enzyme-linked immunosorbent assay
FCS	fully conditional specification
FU	follow-up
FU Period	The period from the EAP visit through the final follow up visit.
FDA	food and drug administration
GCP	good clinical practice
GMT	geometric mean titer
GMFI	geometric mean fold increase
Group 1	The group of subjects seronegative for SARS-CoV-2 antibodies at baseline, 100 µg dose
Group 2	The group of subjects seropositive for SARS-CoV-2 antibodies at baseline, 100 µg dose
Group 3	The group of subjects seropositive for SARS-CoV-2 antibodies at baseline, 50 µg dose
ICF	informed consent form
ICH	International Conference on Harmonization
Last vaccination	Second vaccination for Group 1 at Visit 4, Week 4. Single boost vaccination for Groups 2 and 3 at Visit 1, Day 1.
LLOD	lower limit of detection
LLOQ	lower limit of quantitation
Max	maximum
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
Min	minimum
mRNA	messenger ribonucleic acid

Abbreviation	Definition
n/N	number
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	principal investigator
PMM	predictive mean matching
PT	Preferred Term
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
SDTM	Study Data Tabulation Model
SFU	spot forming units
SI	Système International d'Unités
SOC	System Organ Class
SPEAC	Safety Platform for Emergency vACcines
ULOQ	upper limit of quantitation
Vaccination Period 1	Group 1 only, the time from the first vaccination to just prior to the second vaccination.
Vaccination Period 2	Group 1 only, the time from the second vaccination to the EAP visit
WHO Drug	World Health Organization Drug Dictionary

Appendix 2: Schedule of Events for Group 1

Visit (V)	SCR	V1	V2	V3	V4	V5	V6	V7/ EAP	FU1
Day / Visit +… Days	-14--1	1	V1 +5-7	V1 +12-16	V1 +28-35	V4 +7-10	V4 +12-16	V4 +28-35	V4 +91-105
Target week	-2	0	1	2	4	5	6	8	17
Informed consent	X								
Check incl./excl. criteria	X	X							
Medical History	X								
Complete physical exam[a] (body height and weight, BMI assessment)	X								
Evaluation of vital signs[a]	X	X	X	X	X	X	X	X	X
Targeted physical exam incl. auscultation of the heart and lung[a]		X	X	X	X	X	X	X	X
ECG[a]	X								
Recording of prior and concomitant medication	X	X	X	X	X	X	X	X	
Counseling on avoidance of pregnancy for WOCBP[b]	X	X			X				
AE/SAE/AESI recording		X	X	X	X	X	X	X	X[c]
Pregnancy test for WOCBP[d]	X	X			X			X	
Obtaining blood for safety lab[a]	X			X			X	X[e]	
SARS-CoV-2 specific antibody test	X								
SARS-CoV-2 infection test (PCR)	X		(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]
Hep-B, HCV, HIV test	X								
Serum collection for antibody testing[g]		X		X	X	X	X	X	X
Collection of PBMC[g]		X				X			
Vaccine administration & subject observation (≥30 minutes)		X			X				
Recording of immediate AEs		X			X				
Handout of memory aid		X			X				
Collection of memory aid[h]			X			X			
Examination of injection site			X			X			

Abbreviations: AE = adverse event; AESI = adverse event of special interest; BMI = body mass index; EAP = end of active phase visit; ECG = electrocardiogram; FU = follow-up; Hep-B = hepatitis-B; HCV = hepatitis-C virus; HIV = human immune deficiency virus; PBMC = peripheral blood mononuclear cells; SAE = serious adverse event; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SCR = Screening; WOCBP = woman of childbearing potential;

X: mandatory; (X): if indicated/if applicable

[a] If clinically indicated, additional safety measures can be taken at any other trial visits or at unscheduled visits. In addition, auscultation of the heart and lungs will be performed to check specifically for signs of any heart condition or respiratory disorders.

[b] Review of acceptable contraceptive methods and recent menstrual history with WOCBP

[c] Only SAEs/AESIs will be collected during the follow-up period (FU1)

[d] At SCR, a serum test must be performed. At other visits, a urine pregnancy test will be performed

[e] Only for subjects who discontinued during the trial and coming for EAP visit to obtain final safety data

[f] At any time during the trial starting 2 weeks after vaccination if clinically indicated, e.g. in the presence of COVID-19 typical symptoms.

[g] Any serum blood or PBMC samples must be taken before vaccination

[h] If symptoms persist at Day 8, daily symptoms and temperature will continue to be measured and documented each day until resolved

Appendix 3: Schedule of Events for Groups 2 and 3

Visit (V)	SCR	V1	V2	V3	V4/ EAP	FU1	FU2	FU3	FU4
Day / Visit +… Days	**-14--1**	**1**	**V1 +7-10**	**V1 +12-16**	**V1 +28-35**	**V1 +91-105**	**V1 +181-195**	**V1 +361-375**	**V1 +722-736**
Target week	**-2**	**0**	**1**	**2**	**4**	**13**	**26**	**52**	**104**
Informed consent	X								
Check incl./excl. criteria	X	X							
Medical History	X								
Complete physical exam[a] (body height and weight, BMI assessment)	X								
Evaluation of vital signs[a]	X	X	X	X	X	X			
Targeted physical exam incl. auscultation of the heart and lung[a]		X	X	X	X	X			
ECG[a]	X								
Recording of prior and concomitant medication	X	X	X	X	X	X			
Counseling on avoidance of pregnancy for WOCBP[b]	X	X							
AE/SAE/AESI recording		X	X	X	X	X[c]	X[c]	X[c]	X[c]
Pregnancy test for WOCBP[d]	X	X			X				
Obtaining blood for safety lab[a]	X			X	X[e]				
SARS-CoV-2 specific antibody test	X								
SARS-CoV-2 infection test (PCR)	X		(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]
Hep-B, HCV, HIV test	X								
Serum collection for antibody testing[f]		X	X	X	X	X	X	X	X
Collection of PBMC[g]		X	X						
Vaccine administration & subject observation (≥30 minutes)		X							
Recording of immediate AEs		X							
Handout of memory aid		X							
Collection of memory aid[h]			X						
Examination of injection site			X						

Abbreviations: AE = adverse event; AESI = adverse event of special interest; BMI = body mass index; EAP = end of active phase visit; ECG = electrocardiogram; FU = follow-up; Hep-B = hepatitis-B, HCV = hepatitis C virus; HIV = human immune deficiency virus; PBMC = peripheral blood mononuclear cells; SAE = serious adverse event; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SCR = Screening; WOCBP = woman of childbearing potential.

X: mandatory; (X): if indicated/if applicable

[a] If clinically indicated, additional safety measures can be taken at any other trial visits or at unscheduled visits. In addition, auscultation of the heart and lungs will be performed to check specifically for signs of any heart condition or respiratory disorders.

[b] Review of acceptable contraceptive methods and recent menstrual history with WOCBP

[c] Only SAEs/AESIs will be collected during the follow-up period (FU1-FU4)

[d] At SCR, a serum test must be performed. At other visits, a urine pregnancy test will be performed.

[e] Only for subjects who discontinued during the trial and coming for EAP visit to obtain final safety data

[f] At any time during the trial starting 2 weeks after vaccination if clinically indicated, e.g. in the presence of COVID-19 typical symptoms.

[g] Any serum blood or PBMC samples must be taken before vaccination

[h] If symptoms persist at Day 8, daily symptoms and temperature will continue to be measured and documented each day until resolved.

References

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