Protocol C4591031 – Substudy D

A PHASE 3 MASTER PROTOCOL TO EVALUATE ADDITIONAL DOSE(S) OF BNT162b2 IN HEALTHY INDIVIDUALS PREVIOUSLY VACCINATED WITH BNT162b2 – SUBSTUDY D

Statistical Analysis Plan
(SAP)

Version: 4

Date: 28 Sep 2023

[redacted]

TABLE OF CONTENTS

LIST OF TABLES

APPENDICES

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1/ 17 Feb 2022	6 08 Feb 2022	N/A	N/A
2/ 05 Apr 2022	8 31 Mar 2022	To allow for the collection of data after a dose of BNT162b2 OMI at Visit 404 in participants that received 3 prior doses of BNT162b2 and a fourth dose of either BNT162b2 or BNT162b2 OMI.	• In the Study Design section, the second vaccination of BNT162b2 OMI administered to Group 3 and 4 participants was added. • Table 2 was updated to cover the safety and immunogenicity of the additional dose. • The AE reporting period (in Section 3.1.14) was updated due to the additional dose of BNT162b2 in Cohort 2.
		To allow participants to add the vaccination details to their national vaccination record cards.	• Added text in the footnote of Table 3 to allow Cohort 2 to be unblinded at Visit 404.
		To align with CBER feedback.	• Updated the primary and secondary objectives with respect to demonstrating the noninferiority of seroresponse rate and updated the definition of seroresponse. • Updated the hypotheses, success criteria, and multiplicity strategy.
		To provide the analysis method for GMRs of SARS-CoV-2 VOC-neutralizing titers to reference-strain–neutralizing titers.	• Added Section 5.2.2.1.2 for Ratio of Different Assays Within Same Set of Participants.
		To be more specific in the subgroup analysis.	• Added ethnicity and baseline SARS-CoV-2 status in Section 6.4 for the subgroup analysis.
		Baseline SARS-CoV-2 status is an important indicator in the analysis.	• Added baseline SARS-CoV-2 status as one of the demographic characteristics in Section 6.5.1.1.
		To correct a typographical error in the previous version.	• In Section 6.3.1.3 bullet point 2, “anti-” was removed from “anti-VOC neutralizing titers,” as the prefix “anti-” in this context is extraneous.

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
3/ 03 Feb 2023	11 20 Sep 2022	Protocol clarification.	• In Section 2.3 and Table 3, clarified that participants in Cohort 3 will receive BNT162b2 rather than BNT162b2 OMI as their third dose. • In Table 2, added Group 5 for assessing the safety and tolerability profile of BNT162b2 given as a third dose.
			• In Table 2, the estimands of the primary and secondary objectives for Cohort 3 have been revised to include those with or without serological or virological evidence of past SARS-CoV-2 infection due to high COVID-19 infection rate in this cohort.
		In response to feedback received from CBER.	• In Table 2, added a footnote for Cohort 3; confirmed that participants will not be age-matched to participants in C4591001 but will include a similar percentage of participants with a positive SARS-CoV-2 infection status at baseline as Group 5 of this substudy (Substudy D), whenever feasible. • Updated Group 5 immunogenicity objectives and clarified the estimands to include those with or without serological or virological evidence of past SARS-CoV-2 infection. • These updates regarding Group 5 were also made in Sections 3.1.2, 3.2.1, 6.1.2.4, 6.2.1, and 6.2.2.
		The safety follow-up period is 6 months after the last dose; therefore, a longer study duration is not required.	• In Section 2.3, confirmed that participants in Cohort 1 and Cohort 2 will only be followed for 6 months after their last study vaccination. • In Section 7.2, deleted the safety and immunogenicity analysis 12 months after the last study vaccination.
			• Deleted “Confirmed e-diary errors will be excluded from the analysis” in Section 6.1.1.1.1.
		This will enable the team to make decisions for the program based on the available data.	• In Section 7.2, added additional analysis time points once the sponsor is unblinded to the substudy.

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
4/ 28 Sep 2023	12 20 Sep 2023	To expedite the remaining testing and to focus on the analyses of interest.	In Table 2: • Changed superiority and noninferiority hypotheses G1vG2bA and G2vsG2bA to be descriptive. • Removed "super" superiority hypotheses G1vG2bB and G2vG2bB. • Removed the original objective G5B on noninferiority of anti-Omicron immune response to anti–reference-strain immune response, renamed G5C to G5B. • Specified the reduced analysis time points for exploratory objectives for the immune response.
			In Section 2.3: • Added the overall rationale for protocol amendment 12.
			In Section 3.2.1 and Section 6.2.1: • Removed secondary endpoints corresponding to objectives of G1vG2bB, G2vG2bB, and G5B. • Renamed G5C to G5B.
			In Section 3.3.1: • Changed "at each time point" to "at selected time points."
			• In Section 6.2.1, removed G1vG2bB and G2vG2bB. Renamed G5C to G5B.
		Clarified the consolidation of reactogenicity events recorded on the AE CRF with e-diary data for the reactogenicity summary.	• Added details in Section 3.1.1.1, Section 3.1.1.2, Section 6.1.1.1.1, and Section 6.1.1.2.1.

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
		There is no statistical hypothesis in Cohort 1.	• In Section 5.1.1, clarified that the statistical hypothesis only applies to Cohort 2 and Cohort 3. • In Section 5.1.2, clarified that there is no multiplicity adjustment needed for Cohort 1. • In Section 6.1.2.1.1, removed the hypothesis language and clarified that all statistics are to be descriptive, and that there is no statistical hypothesis for Cohort 1. • In Section 6.1.2.1.1, changed the analysis population to be participants with evidence of past SARS-CoV-2 infection. • In Section 6.1.2.2.1, removed the hypothesis language and clarified that all statistics are to be descriptive, and that there is no statistical hypothesis for Cohort 1.
		The original objective G5B was removed.	• In Section 5.1.1, removed the language on noninferiority tests. • Removed Section 6.2.2.
		To clarify information on the analysis population.	• In Section 6.1.2.3.1, added, "Only participants complying with the key protocol criteria (evaluable participants) and no serological or virological evidence (up to 1 month after receipt of the first dose of study intervention) of past SARS-CoV-2 infection will be included."
		To clarify the analysis time points for exploratory objectives.	• In Section 6.3.1.1, added detailed analysis time points.
		To align with DMB02-GSOP-RF02 7.0 Statistical Analysis Plan Template 31-Jan-2022	• Added Section 7.2 Interim Analyses and Summaries. • Made "Analysis Timing" as Section 7.1.1.

2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study C4591031 – Substudy D. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

2.1. Modifications to the Analysis Plan Described in the Protocol

Not applicable.

2.2. Study Objectives, Endpoints, and Estimands

The estimands corresponding to each primary, secondary, and exploratory objective are described in Table 2.

In the primary safety objective evaluations, missing AE start dates will be imputed according to Pfizer safety rules (Section 5.3). No other missing information (eg, missing e-diary data) will be imputed in the safety analysis.

The estimands to evaluate the immunogenicity objectives are based on the evaluable immunogenicity population (see Section 4 for definition). These estimands estimate vaccine effect in the hypothetical setting where participants follow the study schedules and protocol requirements as directed. Missing serology results will not be imputed. Immunogenicity results that are below the LLOQ, denoted as BLQ, will be set to 0.5 × LLOQ in the analysis; this may be adjusted once additional data on the assay characteristics become available.

Table 2. List of Primary, Secondary, and Exploratory Objectives, Endpoints, and Estimands

Objectives	Estimands	Endpoints
Primary Safety		
• To describe the safety and tolerability profile of BNT162b2 OMI given as the third, fourth, or fifth dose to BNT162b2-experienced participants, or as a 2-dose series to COVID-19 vaccine–naïve participants • To describe the safety and tolerability profile of BNT162b2 given as a third or fourth dose to BNT162b2-experienced participants (Group 2b, Group 4, and Group 5)	In participants receiving at least 1 dose of study intervention, the percentage of participants reporting: • Local reactions for up to 7 days following each dose • Systemic events for up to 7 days following each dose • AEs from the first study vaccination (received in this study) through 1 month after the last study vaccination • SAEs from the first study vaccination (received in this study) through 6 months after the last study vaccination	• Local reactions (pain at the injection site, redness, and swelling) • Systemic events (fever, fatigue, headache, chills, vomiting, diarrhea, new or worsened muscle pain, and new or worsened joint pain) • AEs • SAEs

Table 2. List of Primary, Secondary, and Exploratory Objectives, Endpoints, and Estimands

Objectives	Estimands	Endpoints
Primary Immunogenicity ***BNT162b2-experienced participants***		
G1vG2bA: To descriptively compare the anti-Omicron immune response after 1 dose of BNT162b2 OMI to the immune response after 1 dose of BNT162b2 given as the third dose in BNT162b2-experienced participants	In participants complying with the key protocol criteria (evaluable participants) and with evidence of past SARS-CoV-2 infection: • GMR of the Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI to those at 1 month after 1 dose of BNT162b2 given as the third dose in BNT162b2-experienced participants • The difference in percentages of participants with seroresponse[a] to the Omicron strain at 1 month after 1 dose of BNT162b2 OMI and at 1 month after 1 dose of BNT162b2 given as the third dose in BNT162b2-experienced participants	SARS-CoV-2 Omicron-neutralizing titers
G2vG2bA: To descriptively compare the anti-Omicron immune response after 2 doses of BNT162b2 OMI given as the third and fourth doses to the immune response after 1 dose of BNT162b2 given as the third dose in BNT162b2-experienced participants	In participants complying with the key protocol criteria (evaluable participants) and with evidence of past SARS-CoV-2 infection: • GMR of the Omicron-neutralizing titers at 1 month after 2 doses of BNT162b2 OMI to those at 1 month after 1 dose of BNT162b2 given as the third (and fourth) dose in BNT162b2-experienced participants • The difference in percentages of participants with seroresponse[a] to the Omicron strain at 1 month after 2 doses of BNT162b2 OMI and at 1 month after 1 dose of BNT162b2 given as the third (and fourth) dose in BNT162b2-experienced participants	SARS-CoV-2 Omicron-neutralizing titers

Table 2. List of Primary, Secondary, and Exploratory Objectives, Endpoints, and Estimands

Objectives	Estimands	Endpoints
G3vG4A: To demonstrate the superiority with respect to the level of neutralizing titers and the noninferiority with respect to the seroresponse rate of the anti-Omicron immune response after 1 dose of BNT162b2 OMI compared to after 1 dose of BNT162b2 given as the fourth dose in BNT162b2-experienced participants	In participants complying with the key protocol criteria (evaluable participants) and no serological or virological evidence (up to 1 month after receipt of 1 dose of study intervention) of past SARS-CoV-2 infection: • GMR of the Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI to those at 1 month after 1 dose of BNT162b2 given as the fourth dose in BNT162b2-experienced participants • The difference in percentages of participants with seroresponse[a] to the Omicron strain at 1 month after 1 dose of BNT162b2 OMI and at 1 month after 1 dose of BNT162b2 given as the fourth dose in BNT162b2-experienced participants	SARS-CoV-2 Omicron-neutralizing titers
COVID-19 vaccine–naïve participants		
G5A: To demonstrate the superiority with respect to the level of neutralizing titers and the noninferiority with respect to the seroresponse rate of the anti-Omicron immune response after 2 doses of BNT162b2 OMI compared to after 2 doses of BNT162b2 in participants[b] selected from the C4591001 study	In participants complying with the key protocol criteria (evaluable participants) and with or without serological or virological evidence (up to 1 month after receipt of the second dose of BNT162b2 OMI or BNT162b2 as appropriate) of past SARS-CoV-2 infection: • GMR of the Omicron-neutralizing titers 1 month after the second dose of BNT162b2 OMI to 1 month after the second dose of BNT162b2 in participants selected from the C4591001 study • The difference in percentages of participants with seroresponse[a] to the Omicron strain at 1 month after the second dose of BNT162b2 OMI and at 1 month after the second dose of BNT162b2 in participants selected from the C4591001 study	SARS-CoV-2 Omicron-neutralizing titers

Table 2. List of Primary, Secondary, and Exploratory Objectives, Endpoints, and Estimands

Objectives	Estimands	Endpoints
Secondary Immunogenicity BNT162b2-experienced participants		
G3vG4B: To demonstrate the "super" superiority of the anti-Omicron immune response after 1 dose of BNT162b2 OMI compared to after 1 dose of BNT162b2 given as the fourth dose in BNT162b2-experienced participants	Same as the GMR estimand of G3vG4A	Same as G3vG4A
COVID-19 vaccine–naïve participants		
G5B: To demonstrate the "super" superiority of the anti-Omicron immune response after 2 doses of BNT162b2 OMI compared to after 2 doses of BNT162b2 in participants[b] selected from the C4591001 study	Same as the GMR estimand of G5A	Same as G5A
Exploratory		
To describe the immune response to BNT162b2 OMI or BNT162b2 given as the third and/or fourth and/or fifth dose in BNT162b2-experienced participants	For Cohort 1: At baseline, 1 month after the first study vaccination, and 1 month after the second study vaccination (Group 2 only); and for Cohort 2: At baseline, 1 month and 3 months after the first study vaccination, and 1 month, 3 months, and 6 months after the last study vaccination: • GMT at each time point • GMFRs from before the first dose of study intervention to subsequent time points • Percentages of participants with seroresponse[a] at each time point	• SARS-CoV-2 Omicron-neutralizing titers • SARS-CoV-2 reference-strain–neutralizing titers
To describe the immune response to BNT162b2 OMI in COVID-19 vaccine–naïve participants	At baseline, 1 month and 6 months after Dose 2, and 1 month and 6 months after Dose 3: • GMT at each time point • GMFRs from before the first dose of BNT162b2 OMI to subsequent time points • Percentages of participants with seroresponse[a] at each time point	• SARS-CoV-2 Omicron-neutralizing titers • SARS-CoV-2 reference-strain–neutralizing titers

Table 2. List of Primary, Secondary, and Exploratory Objectives, Endpoints, and Estimands

Objectives	Estimands	Endpoints
To describe the immune response to the reference strain and VOCs in a subset of 30 participants[c] per group		• SARS-CoV-2–neutralizing titers for the reference strain and VOCs
To describe the immune response to any VOCs not already specified (eg, Delta)		• SARS-CoV-2–neutralizing titers for any VOCs not already specified (eg, Delta)
To describe confirmed COVID-19 and severe COVID-19 cases		• Confirmed COVID-19 cases • Confirmed severe COVID-19 cases • Strain sequencing of COVID-19 cases
To describe the cell-mediated immune response, and additional humoral immune response parameters, to the reference strain and Omicron in a subset of participants with PBMC samples collected in each group		

a. Seroresponse is defined as achieving a ≥4-fold rise from baseline (before the first dose of study vaccination). If the baseline measurement is below the LLOQ, the postvaccination measure of ≥4 × LLOQ is considered seroresponse.
b. A subset of approximately 175 participants (18 through 55 years of age) from Study C4591001 who have received 2 doses of BNT162b2 30 µg will be selected for this objective. The subset selected from Study C4591001 will include a similar percentage of participants with a positive SARS-CoV-2 infection status at baseline as Group 5 of this substudy, whenever feasible.
c. This subset of participants will not contribute to the assessment of primary and secondary immunogenicity objectives.

2.3. Study Design

This is a randomized substudy composed of open-labeled and observer-blinded groups to evaluate the safety, tolerability, and immunogenicity of a 2-dose primary series of BNT162b2 OMI, and as a booster (third, fourth, or fifth) dose at investigator sites in the US and South Africa only. Participants ≥18 years of age to ≤55 years of age will be enrolled. Approximately 1420 participants will be enrolled in the study.

Participants in Cohort 1 will have completed a 2-dose primary series of BNT162b2 (30-µg doses), with their last dose 90 to 240 days prior to enrollment. Approximately 615 participants will be randomized at a ratio of 1:1:1 either to receive 1 dose (third) of BNT162b2 OMI, 2 doses (third and fourth) of BNT162b2 OMI, 4 weeks apart, or 1 dose (third) of BNT162b2. Randomization will be stratified by age (stratified as 18-30 and 31-55 years of age). Participants in Cohort 1 will remain blinded to whether they will be receiving a fourth dose through 1 month after their first dose, but the investigator and sponsor will not be blinded.

Participants in Cohort 2 will be enrolled from Study C4591001 and C4591031 Substudy A and will have completed a 2-dose primary series and received a single booster (third) dose of BNT162b2, with their last dose 90 to 180 days prior to randomization. Approximately 600 participants will be randomized at a ratio of 1:1 to receive a fourth dose of either BNT162b2 or BNT162b2 OMI at Visit 401. Participants will be offered a dose of BNT162b2 OMI at Visit 404 (3-month follow-up). Randomization will be stratified by age (stratified as 18-30 and 31-55 years of age). Cohort 2 will be observer blinded.

In Cohort 3, 205 participants 18 through 55 years of age who are COVID-19 vaccine–naïve and have not experienced COVID-19 will be enrolled to receive 2 doses (primary series) of BNT162b2 OMI, 3 weeks apart, with a dose of BNT162b2 approximately 5 months later. If participants do not consent to receive BNT162b2 as a third dose, they will not receive a third dose. No participants should receive BNT162b2 OMI as a third dose.

A subset of 30 participants may be selected from each group to serve as a sentinel group for immunogenicity assessment. Participants in the subset will not contribute to the assessment of primary and secondary immunogenicity objectives. Immunogenicity data from these participants will be summarized separately for the exploratory objective specific for the subset.

Table 3 details the number of participants by cohort and group, their prior BNT162b2 experience, the vaccine that will be administered, and the number of doses administered as part of Substudy D.

Table 3. Total Number of Participants by Cohort

Cohort	Group	Prior BNT162b2 Experience	Vaccine	Number of Doses Administered as Part of Substudy D	Total Number of Participants
Cohort 1	Group 1	2 Doses	BNT162b2 OMI	1	205
	Group 2	2 Doses	BNT162b2 OMI	2	205
	Group 2b	2 Doses	BNT162b2	1	205
Cohort 2	Group 3	3 Doses	BNT162b2 OMI	1 or 2	300
	Group 4	3 Doses	BNT162b2 (and BNT162b2 OMI at Visit 404)	1 or 2	300
Cohort 3	Group 5	Naïve	BNT162b2 OMI	2	205
			BNT162b2	1	

Note: Cohorts 1 and 2 are observer-blinded. Participants in Cohort 1 will remain blinded to whether they will be receiving a fourth dose through 1 month after their first dose of BNT162b2 OMI. Cohort 2 participants will be unblinded once they have completed Visit 404. Cohort 3 is open labeled.

As part of C4591031 protocol amendment 11, active participants in Cohort 1 and Cohort 2 at the time of this amendment will only be followed for 6 months after their last study vaccination, the protocol-specified safety reporting period. Their final visit will be Visit 405.

As part of protocol amendment 12, endpoints and/or analyses that do not pertain to current variant-modified versions of BNT162b2 were removed to expedite the remaining testing and to focus on analyses of interest.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

3.1.1. Primary Safety Endpoints

The primary safety endpoints are as follows:

- Local reactions (redness, swelling, and pain at the injection site) for up to 7 days after each vaccination
- Systemic events (fever, fatigue/tiredness, headache, chills, vomiting, diarrhea, new or worsened muscle pain, and new or worsened joint pain) for up to 7 days after each vaccination
- AEs from the first study vaccination (received in this study) through 1 month after the last study vaccination

- SAEs from the first study vaccination (received in this study) through 6 months after the last study vaccination

3.1.1.1. Local Reactions

The local reactions assessed and reported in the e-diary are redness, swelling, and pain at the injection site, within 7 days after each vaccination. The e-diary entries from the participants and unplanned clinical assessments within 7 days after vaccination will be the primary data sources for these events. In addition, any events recorded on the AE CRF that are considered local reactions within 7 days after vaccination will be consolidated with the e-diary data and included in the reactogenicity report. This section describes derivations with details for the assessment of local reactions: presence, severity level, duration, and onset day.

Presence or Absence

For each local reaction and any local reaction on any day, Table 4 defines the algorithm to derive the presence of a reaction (yes or no) during the interval within 7 days after each dose.

Table 4. Derived Variables for Presence of Each and Any Local Reaction Within 7 Days for Each Vaccination

Variable	Yes (1)	No (0)
Presence of each local reaction on any day	Participant reports the reaction as "yes" on any day (within 7 days after vaccination).	Participant reports the reaction as "no" on all 7 days (after vaccination) or as a combination of "no" and missing on all 7 days (after vaccination).
Presence of any local reaction on any day	Participant reports any local reaction as "yes" on any day (within 7 days after vaccination).	For all 3 local reactions, participant reports "no" on all 7 days (after vaccination) or a combination of "no" and missing on all 7 days (after vaccination).

Note: Missing e-diary data will not be imputed. Participants with no e-diary data reported will not be included in the e-diary summaries.

Severity and Maximum Severity

Redness and swelling will be measured and recorded in measuring device units (range: 1 to 21) and then categorized during analysis as absent, mild, moderate, or severe based on the grading scale in Table 5. Measuring device units can be converted to centimeters according to the following formula: 1 measuring device unit = 0.5 cm. Pain at the injection site will be assessed by the participant as mild, moderate, or severe according to the grading scale in Table 5.

For events recorded on the AE CRF that are considered local reactions and consolidated with the e-diary data, the severity will be based on the AE intensity grade recorded on the CRF.

Table 5. Local Reaction Grading Scale

Local Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[a]
Pain at the injection site	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room visit or hospitalization for severe pain
Redness	>2.0 cm to 5.0 cm (5 to 10 measuring device units)	>5.0 cm to 10.0 cm (11 to 20 measuring device units)	>10 cm (≥21 measuring device units)	Necrosis or exfoliative dermatitis
Swelling	>2.0 cm to 5.0 cm (5 to 10 measuring device units)	>5.0 cm to 10.0 cm (11 to 20 measuring device units)	>10 cm (≥21 measuring device units)	Necrosis

a. Only an investigator or qualified designee is able to classify a participant's local reaction as Grade 4, after clinical evaluation of the participant or documentation from another medically qualified source (eg, emergency room or hospital record). Grade 4 local reactions will be collected on the AE case report form and assessed by the investigator using the AE intensity grading scale.

If a Grade 3 local reaction is reported in the reactogenicity e-diary, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person is able to classify a participant's local reaction as Grade 4. If a participant experiences a confirmed Grade 4 local reaction, the investigator must immediately notify the sponsor and, if it is determined to be related to the administration of the study intervention, further vaccinations will be discontinued in that participant.

For each local reaction after each vaccination, the maximum severity grade will be derived for the e-diary collection period (within 7 days after each vaccination) as follows:

> Maximum severity grade = highest grade (maximum severity) within 7 days after vaccination among the severity grades reported for that local reaction in the e-diary.

Duration (First to Last Day Reported)

The duration (days) of each local reaction will be calculated as the number of days from the start of the first reported reaction to the resolution of the last reported reaction, inclusive. Resolution is defined as the last day on which the reaction is recorded in the e-diary if the reaction lasts 7 days or less, or the day the reaction ends if it persists beyond the end of the reactogenicity e-diary period following vaccination (the latter will be collected on the CRF). If there is no known date when the reaction ended, then duration will be missing (unknown). However, if a reaction is ongoing at the time of a subsequent vaccination, the end date/day for the ongoing event would be the date/day that the next vaccination is administered, which will be used for the duration computation. Participants with no reported reaction have no duration.

If a local reaction is captured in more than 1 data source, eg, the e-diary, an unplanned assessment, and/or the AE CRF, the highest grade (maximum severity) across all sources will be used in the summary.

Onset Day

The onset day of each local reaction will be derived. Onset day is defined as the first day of reporting the reaction with any severity after vaccination.

For the onset day of each local reaction, if participants report a change in severity of the local reaction, only the first day of reporting that specific local reaction will be counted.

3.1.1.2. Systemic Events (Systemic Event Symptoms and Fever)

The systemic events assessed and recorded in the e-diary are fever, fatigue, headache, chills, vomiting, diarrhea, new or worsened muscle pain, and new or worsened joint pain within 7 days after each vaccination. The e-diary entries from the participants and unplanned clinical assessments within 7 days after vaccination will be the primary data sources for these events. In addition, any events recorded on the AE CRF that are considered systemic events starting within 7 days after vaccination will be consolidated with the e-diary data and included in the reactogenicity report. The derivations for systemic events will be handled similarly to the way local reactions are handled for presence of event, severity level, duration, and onset day (see Section 3.1.1.1).

The systemic events will be assessed by the participant as mild, moderate, or severe according to the grading scale in Table 6 and recorded in the e-diary. For events recorded on the AE CRF that are considered systemic events and consolidated with the e-diary data, the severity will be based on the AE intensity grade recorded on the CRF.

If a Grade 3 systemic event is reported in the reactogenicity e-diary, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person is able to classify a participant's systemic event as Grade 4. If a participant experiences a confirmed Grade 4 systemic event, the investigator must immediately notify the sponsor and, if it is determined to be related to the administration of the study intervention, further vaccinations will be discontinued in that participant.

Table 6. Systemic Event Grading Scale

Systemic Event	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Vomiting	1-2 times in 24 hours	>2 times in 24 hours	Requires IV hydration	Emergency room visit or hospitalization for hypotensive shock

Table 6. Systemic Event Grading Scale

Systemic Event	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Diarrhea	2 to 3 loose stools in 24 hours	4 to 5 loose stools in 24 hours	6 or more loose stools in 24 hours	Emergency room visit or hospitalization for severe diarrhea
Headache	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe headache
Fatigue/tiredness	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe fatigue
Chills	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe chills
New or worsened muscle pain	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe new or worsened muscle pain
New or worsened joint pain	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe new or worsened joint pain

Abbreviation: IV = intravenous.

During the 7 days following each vaccination, potential COVID-19 symptoms that overlap with solicited systemic events (ie, fever, chills, new or increased muscle pain, diarrhea, vomiting) should be assessed by the investigator.

If, in the investigator's opinion, the symptoms are considered more likely to be vaccine reactogenicity, but a participant is required to demonstrate that he or she is SARS-CoV-2–negative, a local SARS-CoV-2 test may be performed: if positive, the symptoms should be recorded as an AE rather than as systemic events in the reactogenicity e-diary.

Potential COVID-19 symptoms that do not overlap with systemic events should be reported as AEs as per Section 8.3 of the protocol.

Oral temperature will be collected in the reactogenicity e-diary in the evening daily during the reactogenicity e-diary reporting period (7 days after each vaccination) and at any time during the 7 days that fever is suspected. Fever is defined as an oral temperature of ≥38.0°C (≥100.4°F). The highest temperature for each day will be recorded in the e-diary.

Temperatures will be measured and recorded to 1 decimal place. Temperatures recorded in degrees Fahrenheit will be programmatically converted to degrees Celsius first for reporting. Temperatures <35.0°C and >42.0°C will be excluded from the analysis. Fever will be grouped into ranges for the analysis according to Table 7.

If a fever of ≥39.0°C (≥102.1°F) is reported in the reactogenicity e-diary, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person is able to confirm a participant's fever as >40.0°C (>104.0°F). If a participant experiences a confirmed fever >40.0°C (>104.0°F), the investigator must immediately notify the sponsor and, if it is determined to be related to the administration of the study intervention, further vaccinations will be discontinued in that participant. If a fever is reported on the AE CRF within 7 days after vaccination but the temperature was not recorded, the fever will be included in the reactogenicity summary with "unknown" for temperature range.

Table 7. Scale for Fever

≥38.0-38.4°C (100.4-101.1°F)
>38.4-38.9°C (101.2-102.0°F)
>38.9-40.0°C (102.1-104.0°F)
>40.0°C (>104.0°F)

If a systemic event is captured in more than 1 data source, eg, the e-diary, an unplanned assessment, and/or the AE CRF, the highest grade (maximum severity) across all sources will be used in the summary.

3.1.1.3. Use of Antipyretic Medication

The use of antipyretic medication to treat symptoms associated with study intervention administration will also be recorded in the reactogenicity e-diary daily during the reporting period (7 days after each vaccination). For the use of antipyretic medication within 7 days after each vaccination, the following endpoints and variables will be derived for analysis following the same rules as for local reactions (see Section 3.1.1.1), where applicable.

- Presence (yes or no) of use of antipyretic medication on each day (7 days after each vaccination)
- Presence (yes or no) of use of antipyretic medication on any day (within 7 days after each vaccination)
- Duration (first to last day reported) of use of antipyretic medication
- Onset day of use of antipyretic medication

The use of antipyretic medication will be summarized and included in the systemic event summary tables but will not be considered a systemic event.

3.1.1.4. Adverse Events

AEs will be collected from the time of informed consent through 1 month after the last study vaccination for Cohort 1. For Cohort 2, AEs will be collected from the time of informed consent through 1 month after the first study vaccination and from the second study vaccination through 1 month after the second study vaccination. For Cohort 3, AEs from the time of informed consent through 1 month after the second study vaccination and from the third study vaccination through 1 month after the third study vaccination will be collected. In addition, any AEs occurring up to 48 hours after any subsequent blood draw must be recorded on the CRF. AEs will be categorized according to MedDRA terms. Missing AE start dates will be imputed following the Pfizer data standard rules as described in Section 5.3.

The primary safety endpoint "AEs from the first study vaccination (received in this study) through 1 month after the last study vaccination" and other AE endpoints will be summarized by system organ class and preferred term.

These primary endpoints will be supported by summaries and/or listings of related AEs, severe AEs, immediate AEs (within the first 30 minutes after each vaccination), and AESIs (defined in Section 8.3.8 of the protocol).

3.1.1.5. Serious Adverse Events

SAEs will also be collected from the time of informed consent through 6 months after the last study vaccination. SAEs will be categorized according to MedDRA terms.

The safety endpoint "SAEs from the first vaccination (received in this study) through 6 months after the last study vaccination" will be summarized by system organ class and preferred term. Additionally, SAEs will be listed.

3.1.2. Primary Immunogenicity Endpoints

- (G1vG2bA) SARS-CoV-2 Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI (Group 1) and those at 1 month after 1 dose of BNT162b2 (Group 2b) given as the third dose in BNT162b2-experienced participants

- (G2vG2bA) SARS-CoV-2 Omicron-neutralizing titers at 1 month after 2 doses of BNT162b2 OMI given as the third and fourth doses (Group 2) and those at 1 month after 1 dose of BNT162b2 given as the third dose (Group 2b) in BNT162b2-experienced participants

- (G3vG4A) SARS-CoV-2 Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI (Group 3) and those at 1 month after 1 dose of BNT162b2 (Group 4) given as the fourth dose in BNT162b2-experienced participants

- (G5A) SARS-CoV-2 Omicron-neutralizing titers at 1 month after the second dose of BNT162b2 OMI (Group 5) in COVID-19 vaccine–naïve participants and those at 1 month after the second dose of BNT162b2 in participants selected from the C4591001 study

3.2. Secondary Endpoints

3.2.1. Secondary Immunogenicity Endpoints

- (G3vG4B) SARS-CoV-2 Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI (Group 3) and those at 1 month after 1 dose of BNT162b2 (Group 4) given as the fourth dose in BNT162b2-experienced participants

- (G5B) SARS-CoV-2 Omicron-neutralizing titers at 1 month after the second dose of BNT162b2 OMI (Group 5) in COVID-19 vaccine–naïve participants and those at 1 month after the second dose of BNT162b2 in participants selected from the C4591001 study

3.3. Other Endpoints

3.3.1. Exploratory Immunogenicity Endpoints

- SARS-CoV-2 Omicron-neutralizing titers at selected time points

- SARS-CoV-2 reference-strain–neutralizing titers at selected time points

- SARS-CoV-2 neutralizing titers for the reference strain and VOCs in a subset of 30 participants per group

- SARS-CoV-2 neutralizing titers for any VOCs not already specified (eg, Delta)

- Confirmed COVID-19 cases

- Confirmed severe COVID-19 cases

- Strain sequencing of COVID-19 cases

- Cell-mediated immune response, and additional humoral immune response parameters, to the reference strain and Omicron in a subset of participants with PBMC samples collected in each group

3.4. Baseline Variables

Measurements or samples collected prior to the first study vaccination in this study period are considered the baseline data for the assessments.

3.4.1. Demographics, Medical History, and Physical Examination

The demographic variables will be collected including date of birth, sex (male or female), race (Black/African American, American Indian, or Alaskan native, Asian, Native Hawaiian or other Pacific Islander, White, multiracial, and not reported), and ethnicity (Hispanic/Latino, non-Hispanic/non-Latino, and not reported). In cases where more than 1 category is selected for race, the participant would be counted under the category "multiracial" for analysis.

Age at the time of the first study vaccination (in years) will be derived based on the participant's birthday. For example, if the vaccination day is 1 day before the participant's 48th birthday, the participant is considered to be 47 years old.

Medical history will be collected and categorized according to the current version (at the time of reporting) of MedDRA.

If the clinical assessment indicates that a physical examination is necessary to comprehensively evaluate the participant at Visit 401 or 501, a physical examination will be performed and any findings will be recorded in the source documents and, if clinically significant, the findings will be recorded on the medical history CRF.

3.4.2. E-Diary Transmission

An e-diary will be considered transmitted if any data for the local reactions, systemic events, or use of antipyretic medication are present for any day. If all data are missing for all the items on the e-diary for all 7 days after vaccination, the e-diary will be considered not transmitted.

3.4.3. Prior/Concomitant Vaccines and Concomitant Medications

The following concomitant medications and vaccinations will be recorded on the CRF:

- All vaccinations received from 28 days prior to study enrollment until 28 days following administration of the last study intervention.

- Prohibited medications listed in the protocol, Section 6.8.1, will be recorded to include start and stop dates, name of the medication, dose, unit, route, and frequency.

Prior and concomitant vaccines and concomitant medications will be coded using the WHO Drug Dictionary.

3.5. Safety Endpoints

Local reactions, systemic events, AEs, and SAEs are described in the Primary Safety Endpoints section (Section 3.1.1).

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Analysis populations are defined for the statistical analysis of safety and immunogenicity results in Table 8. Data for all participants will be assessed to determine if participants meet the criteria for inclusion in each analysis population prior to unblinding and releasing the database, and classifications will be documented per standard operating procedures.

Table 8. Description of the Analysis Sets

Population	Description
Enrolled	All participants who have a signed ICD.
Randomized/assigned	All participants who are assigned a randomization number in the IWR system.
Evaluable immunogenicity	All eligible randomized/assigned participants who receive the first study intervention to which they are randomized (for Groups 1, 2b, 3, and 4) or receive 2 doses of study intervention to which they are randomized or assigned with Dose 2 received within 19 to 42 days after Dose 1 (for Groups 2 and 5), have a valid and determinate immunogenicity result from the blood sample collected within 28 to 42 days after the first study vaccination (for Groups 1, 2b, 3 and 4) or within 28 to 42 days after the second study vaccination (for Groups 2 and 5), and have no other important protocol deviations as determined by the clinician.
All-available immunogenicity	All randomized/assigned participants who receive at least 1 dose of the study intervention with a valid and determinate immunogenicity result after vaccination.
Safety	All participants who receive at least 1 dose of the study intervention.

Important protocol deviations will be determined by the medical monitor. An important protocol deviation is a protocol deviation that, in the opinion of the sponsor's clinician, would materially affect assessment of immunogenicity, eg, participant receipt of a prohibited vaccine or medication that might affect immune response or a medication error with suspected decrease in potency of the vaccine. The sponsor's medical monitor will identify those participants with important protocol deviations that result in exclusion from analysis populations before any unblinded analysis is carried out.

For all the immunogenicity endpoints, the analysis will be based on the evaluable immunogenicity population. An additional analysis may be performed based on the all-available immunogenicity population if there is a ≥10% difference in sample size between the all-available immunogenicity population and the evaluable immunogenicity population. Participants will be summarized according to the vaccine group to which they were randomized. Missing serology data will not be imputed.

The safety analyses are based on the safety population. Participants will be summarized by vaccine group according to the study intervention they actually receive. Missing reactogenicity e-diary data will not be imputed; missing AE start dates will be handled according to the Pfizer safety rules.

5. GENERAL METHODOLOGY AND CONVENTIONS

Methodology for statistical analyses of the data collected in this study is described here. The SAP may modify what is outlined in the protocol where appropriate; however, any major modifications of the primary endpoint definitions or their analyses will also be reflected in a protocol amendment.

The majority of sponsor/Pfizer staff will be blinded to study intervention allocation for Cohort 2. All laboratory testing personnel performing serology assays will remain blinded to the study intervention assigned/received throughout the study. Further details on blinding can be found in the protocol, Section 10.10.6.2.2. The timing for statistical analysis is specified in Section 7.1.1.

5.1. Hypotheses and Decision Rules

5.1.1. Immunogenicity Hypothesis

The primary immunogenicity objectives for Cohort 2 and Cohort 3 are to assess the superiority with respect to the level of neutralizing titers and the noninferiority with respect to the seroresponse rate of the anti-Omicron immune response induced by BNT162b2 OMI relative to the anti-Omicron immune response elicited by BNT162b2. Each primary objective for Cohort 2 and Cohort 3 will be evaluated by the following 2 hypotheses:

- The first null hypothesis (H_0) is

$$H_0: \ln(\mu_1) - \ln(\mu_2) \leq \ln(1) \text{ vs } H_1: \ln(\mu_1) - \ln(\mu_2) > \ln(1)$$

where ln(1) corresponds to a 1-fold margin for superiority and

  - $\ln(\mu_1)$ is the natural log of the geometric mean of SARS-CoV-2 Omicron-neutralizing titers measured 1 month after BNT162b2 OMI;
  - $\ln(\mu_2)$ is the natural log of the geometric mean of SARS-CoV-2 Omicron-neutralizing titers measured 1 month after BNT162b2.

- The second null hypothesis (H_0) is

$$H_0: p_1 - p_2 \leq -0.05 \text{ vs } p_1 - p_2 > -0.05$$

where 5% is the noninferiority margin for seroresponse and

  - p_1 is the percentage of participants with seroresponse to the Omicron strain at 1 month after BNT162b2 OMI;
  - p_2 is the percentage of participants with seroresponse to the Omicron strain at 1 month after BNT162b2.

Seroresponse is defined as achieving a ≥4-fold rise from baseline (before the first dose of study vaccination). If the baseline measurement is below the LLOQ, the postvaccination measure of ≥4 × LLOQ is considered seroresponse.

Superiority based on GMR will be declared if the lower limit of the 2-sided 95% CI for the GMR is greater than 1; noninferiority based on seroresponse will be declared if the lower limit of the 2-sided 95% CI for the difference in percentages of participants with seroresponse is >-5%.

The secondary objectives of "super" superiority will be evaluated using a 1.5-fold margin for GMR. "Super" superiority for GMR will be established if the lower limit of the 2-sided 95% CI for the GMR is greater than 1.5.

5.1.2. Multiplicity Adjustment

The immunogenicity objectives for BNT162b2-experienced participants who completed a 2-dose primary series of BNT162b2 prior to enrollment in this study (Cohort 1), BNT162b2-experienced participants who completed a 2-dose primary series and a booster dose of BNT162b2 prior to enrollment in this study (Cohort 2), and COVID-19 vaccine–naïve participants (Cohort 3) will be evaluated independently. The 3 cohorts (2-dose BNT162b2-experienced, 3-dose BNT162b2-experienced, and COVID-19 vaccine–naïve individuals) are different populations with different objectives. The 3 populations are included in the same study to improve operational efficiency. Therefore, no type I error adjustments will be applied to the immunogenicity assessments of the 3 populations.

No multiplicity adjustment is needed for Cohort 1 as there is no statistical hypothesis in Cohort 1.

For each population in Cohort 2 and Cohort 3, the objectives will be evaluated in sequential order as listed below using a 1-sided alpha of 0.025:

- Cohort 2 (Groups 3 and 4): G3vG4A → G3vG4B
- Cohort 3 (Group 5): G5A → G5B

For objectives involving 2 hypotheses, hypotheses based on GMR and seroresponse rate difference will be assessed sequentially in the order as stated. Both hypotheses within the objective must be established before assessing the next objective in the sequence. Therefore, the overall type I error is fully controlled for each of these 2 populations.

5.2. General Methods

Unless stated otherwise, "vaccine group" in this document refers to Groups 1, 2, 2b, 3, 4, and 5 defined in Table 3.

CIs for all endpoints in the statistical analysis will be presented as 2-sided at the levels of 95% unless specified otherwise.

5.2.1. Analyses for Binary Endpoints

Descriptive statistics for categorical variables (eg, proportions) are the percentage (%), the numerator (n) and the denominator (N) used in the percentage calculation, and the CI where applicable.

The exact CI for binary endpoints for each group will be computed using the F distribution (Clopper-Pearson).[1] The CI for the between-group difference for binary endpoints will be calculated using the Miettinen and Nurminen[2] method.

5.2.2. Analyses for Continuous Endpoints

Unless otherwise stated, descriptive statistics for continuous variables are n, mean, median, standard deviation, minimum, and maximum.

5.2.2.1. Geometric Mean Ratios

5.2.2.1.1. Between-Group Comparison

The GMRs will be calculated as the difference in the means of logarithmically transformed assay results between 2 vaccine groups and exponentiating the difference. The associated 2-sided 95% CIs will be obtained by calculating CIs using Student's t-distribution for the mean difference of the logarithmically transformed assay results and exponentiating the confidence limits.

5.2.2.1.2. Ratio of Different Assays Within Same Set of Participants

The ratio of SARS-CoV-2 VOC-neutralizing titers to reference-strain–neutralizing titers within the same participant will be limited to those with nonmissing values for both titers. The GMRs of SARS-CoV-2 VOC-neutralizing titers to reference-strain–neutralizing titers will be calculated as the mean of the difference of logarithmically transformed assay results and exponentiating the mean. Two-sided CIs will be obtained by calculating CIs using the 1-sample Student's t-distribution for the mean difference of the logarithmically transformed assay results and exponentiating the confidence limits.

5.2.2.2. Geometric Means

The geometric means will be calculated as the mean of the assay results after making the logarithm transformation and then exponentiating the mean to express results on the original scale. Two-sided 95% CIs will be obtained by taking log transforms of assay results, calculating the 95% CI with reference to Student's t-distribution, and then exponentiating the confidence limits.

5.2.2.3. Geometric Mean Fold Rises

GMFRs are defined as ratios of the results after vaccination to the results before vaccination. GMFRs are limited to participants with nonmissing values at both time points.

GMFRs will be calculated as the mean of the difference of logarithmically transformed assay results (later time point minus earlier time point) and exponentiating the mean. The associated 2-sided 95% CIs will be obtained by constructing CIs using Student's t-distribution for the mean difference on the logarithm scale and exponentiating the confidence limits.

5.2.2.4. Reverse Cumulative Distribution Curves

Empirical RCDCs will plot proportions of participants with values equal to or exceeding a specified assay value versus the indicated assay value, for all observed assay values. Data points will be joined by a step function with data points on the left side of the step.

5.3. Methods to Manage Missing Data

A partial AE start date (missing day or missing both month and day) will be imputed by assigning the earliest possible start date using all available information, such as the stop date of the AE and the vaccination date(s) from the same participant, following the Pfizer standard for handling incomplete AE start date. A complete missing start date for an AE is not allowed in the data collection.

Missing serology results will not be imputed. Immunogenicity results that are below the LLOQ will be set to 0.5 × LLOQ in the analysis; this may be adjusted once additional data on the assay characteristics become available.

No additional imputation will be applied to other missing data.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

6.1.1. Primary Safety Endpoints

6.1.1.1. Local Reactions

6.1.1.1.1. Main Analysis

- Estimands: The percentage of participants reporting local reactions (redness, swelling, and pain at the injection site) for up to 7 days after each dose (Section 2.2).
- Analysis set: Safety population Section 4.
- Analysis time point: Up to 7 days after each dose.
- Analysis methodology: Descriptive statistics (Section 5.2.1).
- Intercurrent events and missing data: The participants without any e-diary or unplanned clinical assessment data, and without reactogenicity data reported on the AE CRF throughout the 7 days after vaccination, will be excluded from the analysis at that particular vaccination; missing values will not be imputed.
- Reporting results: Descriptive statistics for each and any local reaction after each dose in each vaccine group will be presented by maximum severity and cumulatively across severity levels. Descriptive summary statistics will include counts and percentages of participants with the indicated endpoint and the associated 2-sided Clopper-Pearson 95% CIs.

6.1.1.1.2. Supplemental Analyses

To support the assessment of local reactions, the following endpoints (as defined in Section 3.1.1.1) will be summarized with the same analysis time point and analysis population as above, and appropriate analysis methodology and reporting results:

- Duration (days) of each local reaction after each dose.
- Onset day of each local reaction after each dose.

These continuous endpoints will be summarized by displaying n, mean, median, standard deviation, minimum, and maximum for each dose by vaccine group.

In addition, the proportions of participants reporting each local reaction after any dose will be summarized by maximum severity level for Groups 2 and 5.

Figures:

Bar charts with the proportions of participants for each local reaction throughout 7 days after each dose will be plotted for each dose by vaccine group. The bars will be divided into severity categories to highlight the proportions of participants by maximum severity.

6.1.1.2. Systemic Events

6.1.1.2.1. Main Analysis

- Estimands: The percentage of participants reporting systemic events (fever, fatigue, headache, chills, vomiting, diarrhea, new or worsened muscle pain, and new or worsened joint pain) for up to 7 days after each dose (Section 2.2).
- Analysis set: Safety population (Section 4).
- Analysis time point: Up to 7 days after each dose.
- Analysis methodology: Descriptive statistics (Section 5.2.1).
- Intercurrent events and missing data: The participants without any e-diary or unplanned clinical assessment data, and without reactogenicity events reported on the AE CRF throughout the 7 days after vaccination, will be excluded from the analysis at that particular vaccination; missing values will not be imputed.
- Reporting results: Descriptive statistics for each systemic event after each dose in each vaccine group will be presented by maximum severity and cumulatively across severity levels. Descriptive summary statistics will include counts and percentages of participants with the indicated endpoint and the associated 2-sided Clopper-Pearson 95% CIs.

6.1.1.2.2. Supplemental Analyses

The following endpoints for assessment of systemic events will be summarized similarly to the assessment of local reactions:

- Duration of each systemic event after each dose.
- Onset day of each systemic event after each dose.

These continuous endpoints will be summarized by displaying n, mean, median, standard deviation, minimum, and maximum for each dose by vaccine group.

The use of antipyretic medication (see Section 3.1.1.3) will be summarized similarly to systemic events, except that there is no severity level associated with the use of antipyretic medication.

In addition, the proportions of participants reporting each prompted systemic event after any dose will be summarized by maximum severity level for Groups 2 and 5.

Figures:

Bar charts with the proportions of participants reporting each systemic event throughout 7 days will be plotted for each vaccine group. The bars will be divided into severity categories to highlight the proportions of participants by maximum severity.

6.1.1.3. Adverse Events

6.1.1.3.1. Main Analysis

- Estimand: The percentage of participants reporting AEs from the first study vaccination (received in this study) through 1 month after the last study vaccination (Cohort 1), within 1 month after each study vaccination (Cohort 2), or from the first study vaccination through 1 month after the second study vaccination and from the third study vaccination through 1 month after the third study vaccination (Cohort 3) (Section 2.2).
- Analysis set: Safety population (Section 4).
- Analysis time points: Refer to the estimand above.
- Analysis methodology: Descriptive statistics (Section 5.2.1 and Section 3.1.1.4).
- Intercurrent events and missing data: Missing data will not be imputed except for partial AE start dates (Section 5.3).
- Reporting results: Counts, percentages, and the associated 2-sided Clopper-Pearson 95% CIs of AEs within 1 month after the last study vaccination (Cohorts 1 and 2) or the second study vaccination (Cohort 3) will be provided for each vaccine group.

6.1.1.3.2. Supplemental Analyses

For Cohort 3, AEs occurring within 1 month after the third study vaccination will also be summarized similarly as described in Section 6.1.1.3.1.

Related AEs, severe AEs, immediate AEs (within the first 30 minutes after each dose), and AESIs (defined in Section 8.3.8 of the protocol) will also be summarized by vaccine group.

All AEs after informed consent and prior to the first vaccination will not be included in the analyses but will be in the listing.

6.1.1.4. Serious Adverse Events

6.1.1.4.1. Main Analyses

- Estimand: The percentage of participants reporting SAEs from the first study vaccination (received in this study) through 6 months after the last study vaccination (Section 2.2).
- Analysis set: Safety population Section 4.
- Analysis time point: From the first study vaccination (received in this study) through 6 months after the last study vaccination.
- Analysis methodology: Descriptive statistics (Section 5.2.1).
- Intercurrent events and missing data: Missing data will not be imputed except for partial AE start dates (Section 5.3).
- Reporting results: Counts, percentages, and the associated Clopper-Pearson 95% CIs of SAEs from the first study vaccination (received in this study) through 6 months after the last study vaccination will be provided for each vaccine group.

6.1.2. Primary Immunogenicity Endpoints

6.1.2.1. Primary Immunogenicity Endpoint for Group 1 vs Group 2b (G1vG2bA)

SARS-CoV-2 Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI (Group 1) and those at 1 month after 1 dose of BNT162b2 (Group 2b) given as the third dose in BNT162b2-experienced participants.

6.1.2.1.1. Main Analyses

- Estimands:
  1. GMR of the Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI (Group 1) to those at 1 month after 1 dose of BNT162b2 (Group 2b) given as the third dose in BNT162b2-experienced participants (Section 2.2).
  2. The difference in percentages of participants with seroresponse to the Omicron strain at 1 month after 1 dose of BNT162b2 OMI (Group 1) and at 1 month after 1 dose of BNT162b2 (Group 2b) given as the third dose in BNT162b2-experienced participants (Section 2.2).
- Analysis set: Evaluable immunogenicity population, all-available immunogenicity population (as applicable) (Section 4).
- Analysis time point: 1 Month after 1 dose of BNT162b2 OMI for Group 1 and 1 month after 1 dose of BNT162b2 for Group 2b.

- Analysis methodology: GMRs and the associated 2-sided 95% CIs will be calculated using the statistical methods described in Section 5.2.2. The percentages of participants with seroresponse for each group will be provided. The difference in percentages of participants with seroresponse and the associated 2-sided 95% CIs will be calculated using the Miettinen and Nurminen method (Section 5.2.1). All statistics will be descriptive and there will be no statistical hypothesis for Cohort 1.

- Intercurrent events and missing data: Serology data deemed unevaluable because of noncompliance with the key protocol criteria will be excluded. Missing data will not be imputed. Only participants who comply with the key protocol criteria (evaluable participants) and have evidence of past SARS-CoV-2 infection will be included.

- Reporting results: The GMTs with the corresponding 95% CIs, GMR, and the associated 2-sided 95% CI will be provided. The numbers/percentages of participants with seroresponse for each group and the corresponding 95% CIs will be provided. The difference in percentages of participants with seroresponse between the 2 groups and the associated 2-sided 95% CIs will be calculated using the Miettinen and Nurminen method.

6.1.2.2. Primary Immunogenicity Endpoint for Group 2 vs Group 2b (G2vG2bA)

- SARS-CoV-2 Omicron-neutralizing titers at 1 month after 2 doses of BNT162b2 OMI given as the third and fourth doses (Group 2) and those at 1 month after 1 dose of BNT162b2 given as the third dose (Group 2b) in BNT162b2-experienced participants.

6.1.2.2.1. Main Analyses

- Estimands:

    1. GMR of the Omicron-neutralizing titers at 1 month after 2 doses of BNT162b2 OMI given as the third and fourth doses (Group 2) to those at 1 month after 1 dose of BNT162b2 given as the third dose (Group 2b) in BNT162b2-experienced participants (Section 2.2).

    2. The difference in percentages of participants with seroresponse to the Omicron strain at 1 month after 2 doses of BNT162b2 OMI given as the third and fourth doses (Group 2) and at 1 month after 1 dose of BNT162b2 given as the third dose (Group 2b) in BNT162b2-experienced participants (Section 2.2).

- Analyses of G2vG2bA are similar to those for G1vG2bA described in Section 6.1.2.1.1.

6.1.2.3. Primary Immunogenicity Endpoint for Group 3 vs Group 4 (G3vG4A)

- SARS-CoV-2 Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI (Group 3) and those at 1 month after 1 dose of BNT162b2 (Group 4) given as the fourth dose in BNT162b2-experienced participants.

6.1.2.3.1. Main Analyses

- Estimands:

    1. GMR of the Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI (Group 3) to those at 1 month after 1 dose of BNT162b2 (Group 4) given as the fourth dose in BNT162b2-experienced participants (Section 2.2).

    2. The difference in percentages of participants with seroresponse to the Omicron strain at 1 month after 1 dose of BNT162b2 OMI (Group 3) and at 1 month after 1 dose of BNT162b2 (Group 4) given as the fourth dose in BNT162b2-experienced participants (Section 2.2).

- Analyses: GMR and the associated 2-sided 95% CIs, the percentages of participants with seroresponse, and the difference in percentages of participants with seroresponse and the associated 2-sided 95% CIs will be calculated using the same method as for G1vG2bA described in Section 6.1.2.1.1. Only participants who comply with the key protocol criteria (evaluable participants) and have no serological or virological evidence (up to 1 month after receipt of the first dose of study intervention) of past SARS-CoV-2 infection will be included. Superiority based on GMR and noninferiority based on seroresponse will be evaluated using the criteria as described in Section 5.1.1.

6.1.2.4. Primary Immunogenicity Endpoint for Group 5 (G5A)

- SARS-CoV-2 Omicron-neutralizing titers at 1 month after the second dose of BNT162b2 OMI (Group 5) in COVID-19 vaccine–naïve participants and those at 1 month after the second dose of BNT162b2 in participants selected from the C4591001 study.

6.1.2.4.1. Main Analyses

- Estimands:

    1. GMR of the Omicron-neutralizing titers at 1 month after the second dose of BNT162b2 OMI (Group 5) in COVID-19 vaccine–naïve participants to those at 1 month after the second dose of BNT162b2 in participants selected from the C4591001 study (Section 2.2).

    2. The differences in percentages of participants with seroresponse to the Omicron strain at 1 month after the second dose of BNT162b2 OMI (Group 5) in COVID-19 vaccine–naïve participants and at 1 month after the second dose of BNT162b2 in participants selected from the C4591001 study (Section 2.2).

- Analyses: Participants complying with the key protocol criteria (evaluable participants) and with or without serological or virological evidence (up to 1 month after receipt of the second dose of BNT162b2 OMI or BNT162b2 as appropriate) of past SARS-CoV-2 infection will be included in the analyses. GMR and the associated 2-sided 95% CIs, the percentages of participants with seroresponse, and the difference in percentages of participants with seroresponse and the associated 2-sided 95% CIs will be calculated using same method as for G1vG2bA (Section 6.1.2.1.1). Superiority based on GMR and noninferiority based on seroresponse will be evaluated using the criteria as described in Section 5.1.1.

6.2. Secondary Endpoints

6.2.1. Secondary Immunogenicity Endpoints for G3vG4B, and G5B ("Super" Superiority)

- Estimands:
  - G3vG4B:

    GMR of the Omicron-neutralizing titers at 1 month after 1 dose of BNT162b2 OMI (Group 3) to those at 1 month after 1 dose of BNT162b2 (Group 4) given as the fourth dose in BNT162b2-experienced participants (Section 2.2).

  - G5B:

    GMR of the Omicron-neutralizing titers at 1 month after the second dose of BNT162b2 OMI (Group 5) in COVID-19 vaccine–naïve participants to those at 1 month after the second dose of BNT162b2 in participants selected from the C4591001 study (Section 2.2).

- Analyses: GMR and the associated 2-sided 95% CIs will be calculated using same method as for G1vG2bB (Section 6.1.2.1.1). "Super" superiority will be evaluated using the criteria as described in Section 5.1.1.

6.3. Exploratory Endpoints

6.3.1. Exploratory Immunogenicity Endpoints

6.3.1.1. SARS-CoV-2 Omicron- or Reference-Strain–Neutralizing Titers

- Estimands:

  1. GMTs of SARS-CoV-2 Omicron- or reference-strain–neutralizing titers at each time point.

  2. GMFRs of SARS-CoV-2 Omicron- or reference-strain–neutralizing titers from baseline (before the first study vaccination received in this study) to each subsequent time point after vaccination(s) for each vaccine group.

3. Percentages of participants with seroresponse to Omicron or the reference strain at each time point.

- Analysis set: Evaluable immunogenicity population (Section 4).
- Analysis time point:

    a. Cohort 1: At baseline, 1 month after the first study vaccination, and 1 month after the second study vaccination (Group 2 only).

    b. Cohort 2: At baseline, 1 month and 3 months after the first study vaccination, and 1 month, 3 months, and 6 months after the last study vaccination.

    c. Cohort 3: At baseline, 1 month and 6 months after Dose 2, and 1 month and 6 months after Dose 3.

- Analysis methodology: The GMTs and the associated 2-sided 95% CIs at each of the above time points will be provided using the statistical methods described in Section 5.2.2.2. The GMFRs and the associated 2-sided 95% CIs from baseline to each of the above postvaccination time points will be provided using the statistical methods described in Section 5.2.2.3. The percentages of participants with seroresponse at each time point and the associated Clopper-Pearson 95% CIs will be provided for each vaccine group (Section 5.2.1).
- Intercurrent events and missing data: Serology data deemed unevaluable because of noncompliance with the key protocol criteria will be excluded. Missing data will not be imputed.
- Reporting results: GMTs at each time point and GMFRs of SARS-CoV-2 Omicron- or reference-strain–neutralizing titers from baseline (before the first study vaccination received in this study) to each subsequent time point after vaccination(s), along with the associated 2-sided 95% CIs, will be provided for each vaccine group. The percentages of participants with seroresponse at each time point and the associated Clopper-Pearson 95% CIs will be provided for each vaccine group.

6.3.1.2. SARS-CoV-2 Neutralizing Titers for the Reference Strain and VOCs for the Subset of 30 Participants per Group

- Estimands:

    1. GMTs of SARS-CoV-2 neutralizing titers for the reference strain and VOCs for the subset of 30 participants per group.

    2. GMFRs of SARS-CoV-2 neutralizing titers for the reference strain and VOCs from baseline (before the first study vaccination received in this study) to each subsequent time point after vaccination for the subset of 30 participants per group.

3. Percentages of participants with seroresponse to the reference strain and VOCs for the subset of 30 participants per group.

- Analysis set: A subset of 30 participants per group.
- Analysis methodology: GMTs, GMFRs, and percentages of participants with seroresponse for the subset of 30 participants per group, along with the associated 95% CIs, will be calculated using same method as described in Section 6.3.1.1.

6.3.1.3. SARS-CoV-2 Neutralizing Titers for Any VOCs Not Already Specified

- Estimands: GMTs for any VOCs not already specified, after any dose of BNT162b2 OMI or BNT162b2.
- Analyses: GMTs of SARS-CoV-2 VOC-neutralizing titers, along with the associated 2-sided 95% CIs, will be provided at specific time points for each group. GMFRs and GMRs of SARS-CoV-2 VOC-neutralizing titers to reference-strain–neutralizing titers may also be calculated along with the associated 2-sided 95% CIs.

6.3.1.4. COVID-19 Cases

Confirmed COVID-19 cases, confirmed severe COVID-19 cases, and strain sequencing of the COVID-19 cases will be summarized.

6.3.1.5. Cell-Mediated Immune Response

The cell-mediated immune response and additional humoral immune response parameters to the reference strain and Omicron will be summarized at each time point for the subset of participants with PBMC samples collected in each group.

6.4. Subset Analyses

Subgroup analyses based on age group, sex, race, ethnicity, and baseline SARS-CoV-2 status (except for Cohort 1 immunogenicity endpoints) will be performed on all primary safety and immunogenicity endpoints (as supplemental analyses).

6.5. Baseline and Other Summaries and Analyses

6.5.1. Baseline Summaries

6.5.1.1. Demographic Characteristics

Demographic characteristics, including age at the first study vaccination, age group, sex, race, ethnicity, baseline SARS-CoV-2 status, and classification of BMI will be summarized using descriptive statistics for each vaccine group based on the safety population and the evaluable immunogenicity population. Timing of previous doses of BNT162b2 prior to enrollment will also be summarized for each vaccine group for Cohorts 1 and 2.

6.5.1.2. Medical History

Each reported medical history term will be mapped to a system organ class and preferred term according to the current version (at the time of reporting) of MedDRA. The number and percentage of participants with at least 1 diagnosis, overall and at each system organ class and preferred term level, will be summarized by vaccine group for the safety population.

6.5.2. Study Conduct and Participant Disposition

6.5.2.1. Participant Disposition

The number and percentage of randomized participants will be included in the disposition summary. In addition, the numbers and percentages of participants who receive vaccinations, who complete the study, and who withdraw from the study (including withdrawals before each planned vaccination visit), along with the reasons for withdrawal, will be tabulated by vaccine group (according to randomized group assignment).
The reasons for withdrawal will be those as specified in the database.

Participants excluded from each analysis population will also be summarized separately along with the reasons for exclusion, by vaccine group.

6.5.2.2. Blood Samples for Assay

The number and percentage of randomized participants providing blood samples within and outside of protocol-prespecified time frames will be tabulated separately for each time point by vaccine group.

6.5.2.3. Transmission of E-Diaries

The number and percentage of vaccinated participants not transmitting the e-diary, transmitting the e-diary for each day, and transmitting the e-diary for all days in the required reporting period for each dose will be summarized according to the vaccine actually received.

The safety population will be used.

6.5.3. Study Intervention Exposure

6.5.3.1. Vaccination Timing and Administration

The number and percentage of participants randomized and receiving each study intervention within the protocol-specified time frame, as well as before and after the specified time frame, will be tabulated for each vaccine group and overall, for all randomized participants. The denominator for the percentage calculations is the total number of randomized participants in the given vaccine group or overall.

A listing of participants showing the randomized vaccine and the vaccine actually received at each vaccination will be presented.

6.5.4. Prior/Concomitant Vaccinations and Concomitant Medications

Each prior/concomitant vaccine will be summarized according to the ATC fourth-level classification. All vaccines received within 28 days before the first study vaccination will be listed. The number and percentage of participants receiving each concomitant vaccine after the first study vaccination will be tabulated by vaccine group. Prohibited medications will be summarized in a similar way as concomitant vaccines. Listings of concomitant vaccines and prohibited medications will be provided. The safety population will be used.

6.6. Safety Summaries and Analyses

6.6.1. Adverse Events

Summaries and analyses of the safety measures, local reactions, systemic events, AEs, and SAEs are described in the Primary Safety Endpoints section (see Section 6.1.1).

7. INTERIM ANALYSES

7.1. Introduction

No formal interim analysis will be conducted for this study. Statistical analyses will be carried out when the final data for specified objectives are available while the study is ongoing. The timing of these planned analysis and reporting events is described in the section below.

7.1.1. Analysis Timing

Statistical analyses will be carried out when the following data are available:

- Safety data through 1 month after the first study vaccination received in this study for Groups 1, 2b, 3, and 4 or through 1 month after the second study vaccination received in this study for Groups 2 and 5.
- Immunogenicity data for the subset of 30 participants per group.
- Immunogenicity data through 1 month after the first study vaccination (for Groups 1, 2b, 3, and 4) or 1 month after the second study vaccination (for Groups 2 and 5) received in this study for participants enrolled in this study and through 1 month after Dose 2 for the comparator group from the C4591001 study.
- Complete safety and immunogenicity analysis approximately 6 months after the last study vaccination for each group.

Certain analyses may be combined as 1 regulatory submission report if the data become available around the same time. Additional analyses may be conducted if required for regulatory purposes. Protocol-specified exploratory analyses may be conducted at additional time points once the sponsor is unblinded to the substudy to provide supporting data to inform decisions at the study and program level. All analyses conducted while the study is ongoing will be performed by an unblinded team.

7.2. Interim Analyses and Summaries

Not applicable.

8. REFERENCES

1. Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika. 1934;26(4):404-13.

2. Miettinen O, Nurminen M. Comparative analysis of two rates. Stat Med. 1985;4(2):213-26.

9. APPENDICES

Appendix 1. List of Abbreviations

Abbreviation	Term
AE	adverse event
AESI	adverse event of special interest
ATC	Anatomic Therapeutic Chemical
BLQ	below limit of quantitation
BMI	body mass index
BNT162b2 OMI	BNT162b2 OMICRON (B.1.1.529)
CBER	Center for Biologics Evaluation and Research
CI	confidence interval
COVID-19	coronavirus disease 2019
CRF	case report form
e-diary	electronic diary
GMFR	geometric mean fold rise
GMR	geometric mean ratio
GMT	geometric mean titer
ICD	informed consent document
IWR	interactive Web-based response
LLOQ	lower limit of quantitation
MedDRA	Medical Dictionary for Regulatory Activities
N/A	not applicable
OMI	Omicron (ie, in BNT162b2 OMI)
PBMC	peripheral blood mononuclear cell
RCDC	reverse cumulative distribution curve
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
US	United States
VOC	variant of concern
WHO	World Health Organization

Document Approval Record

Document Name:	C4591031 Sub Study D Statistical Analysis Plan V4, Clean Copy, 28 Sep 2023
Document Title:	A PHASE 3 MASTER PROTOCOL TO EVALUATE ADDITIONALL DOSE(S) OF BNT162b2 IN HEALTHY INDIVUALS PREVIOUSLY VACCINATED WITH BNT162b2 - SUBSTUDY D Statistical Analysis Plan (SAP)

Signed By:	Date(GMT)	Signing Capacity
PPD	28-Sep-2023 17:04:48	Final Approval