Protocol BNT162-17
Version: 7.0
EudraCT no.: 2021-003458-22
ClinicalTrials.gov: NCT05004181

A PHASE II TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF SARS-COV-2 MONOVALENT AND MULTIVALENT RNA-BASED VACCINES IN HEALTHY SUBJECTS

Statistical Analysis Plan (SAP)

Version: Final 5.0

Date: 23 Feb 2024

Prepared by:

ICON Clinical Research Services

On behalf of:

BioNTech SE

Signature Page

Prepared at ICON Clinical Research by:

PPD, Biostatistician **Date**

Reviewed at ICON Clinical Research by:

PPD, Senior Biostatistician **Date**

Approved at BioNTech SE by:

PPD, Associate Director, Biostatistics **Date**

Approved at BioNTech SE by:

PPD, Senior Medical Director Clinical Development **Date**

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

1 VERSION HISTORY

Table 1. Summary of changes

Version/ date	Associated protocol	Summary and rationale for changes
1/ 17 Jan 2022	Protocol V3.0, 05 Nov 2021	N/A
2/ 30 Jan 2023	Protocol V6.0, 04 Aug 2022	The document is updated to align with the updates made in protocol version 6.0 (CTP V6 04Aug2022). Also, the document is modified to align with regulatory requirements on presentation of summaries and analyses outputs. Minor editorial changes, such as typing errors are not listed here. Throughout the document: • Summaries and analysis for trial Part C is added. • "Systemic reactions" is changed to "systemic events." • Where appropriate, "Study" meaning clinical study is changed to "Trial" meaning clinical trial. • Hyphens in "1 month" removed. • "mcg" is replaced with "µg". • IgG and Biomarkers analysis are removed from the document.
		Section 2.1: • Description of objectives, estimands and endpoints for Part C of this trial are added.
		Section 2.2.1.3: • Trial design for Part C is added.
		Section 3.1.1: • The definition of "Last Dose" for each cohort is added.

Version/ date	Associated protocol	Summary and rationale for changes
		Section 3.1.1.1: • Onset day definition is modified to make it clear that reactogenicity events onset day will be computed separately for each IMP injection. • Maximum duration description is modified to make it clear that reactogenicity events maximum duration computation will be computed separately for each IMP injection.
		Section 3.1.1.3: • AE/SAE section is revised to align with CTP v6.0.
		Section 3.1.2: • The primary immunogenicity endpoints for Part B of the trial are revised as per the CTP v6.0. • The definition of seroresponse after immunization is revised for ease of derivation.
		Section 3.1.3: • The primary immunogenicity endpoints for Part C are included as per the CTP v6.0.
		Section 3.2.1: • The secondary immunogenicity endpoints for Part C are added as per the CTP v6.0.
		Section 3.3: • Exploratory immunogenicity endpoints for Part-C added as per the CTP v6.0. • In case an Exploratory endpoint is also a primary or secondary endpoint for any part of the study A, B or C then it would not be repeated in analysis.
		Section 3.3.1: • Confirmed COVID-19 Cases and the handling of Surveillance Period are updated.
		Section 3.3.2: • "Nucleocapsid-binding antibody seroconversion" and the Surveillance Period are updated.

Version/ date	Associated protocol	Summary and rationale for changes
		Section 3.4: • Baseline value to use in the derivation of SR was modified for clarity. • A derivation for Baseline SARS-CoV-2 infection status was added. • The analysis visit handling for safety analysis and the protocol specified window for Immunogenicity and sensitivity analysis has been updated.
		Section 3.4.1: • The variable "Time at IMP dose 1 since dose 2 of BNT162b2 (days)" and "Childbearing potential" was added.
		Section 3.4.2: • The concomitant medication/procedure part is updated.
		Section 4: • To capture wrongly dosed subjects in the IPD table the analysis set used to generate this table was changed from "Safety set" to the "Screened Set". • Immunogenicity per protocol analysis set was added in Table 8 of Analysis set.
		Section 5: • A sentence was added to make it clear that only reactogenicity events within 7 days will be reported in the analysis and summaries.
		Section 5.1.4.2 and 5.1.4.3: • Updated to reflect the objectives of updates as per CTP v6.0.
		Section 5.2: • "Data assessed after the vaccination with a non-trial vaccine" will not be part of statistical analysis.

Version/ date	Associated protocol	Summary and rationale for changes
		Section 5.3: • A sentence was included to make it clear that missing binary and continuous data will be presented only if there is a non-zero count for at least one of the Cohorts. • For all trial parts, if no data is available to any of the Cohorts, then the output will show "No data to display".
		Section 6.1.1.1: • This section was modified to clarify that local reactions recorded beyond 7 days will be excluded from the analysis.
		Section 6.1.1.3: • A summary table for solicited AE that continues longer than 7 days post vaccination or an SAE or an AE that fits the definition of a solicited AE but starts after Day 7 was included.
		Section 6.1.2: • Sensitivity Analysis will be done updated throughout all the subsections of this part.
		Section 6.2: • The presentation of the bar graphs updated to include LLOQ and ULOQ horizontal lines also color coding to differentiate data from subjects N-binding assessment for the respective visit, time point.
3/ 29 Jun 2023	Protocol V7.0, 28 Jun 2023	Section 2.1, 3.1.2, 3.2.1, 5.1, 6.1.2, 6.2.2: • Add primary and secondary objectives, endpoints, hypotheses, and analyses and summaries for the new objectives in Protocol V7.0.
		Section 3.1.2 • "Value above ULOQ will be set to ULOQ." Section 4.
		• "All Randomized Subjects", "Reactogenicity Set", and "All-available Immunogenicity Set (mITT)" were added in Section 4.

Version/ date	Associated protocol	Summary and rationale for changes
		Section 3.4: • Updated definition of baseline for the primary non-inferiority test of the difference of SR in Part B Cohort 1 and 4 to align with the definition for the descriptive analysis of GMFR and SR. • COVID-19 infection status at baseline and during surveillance are updated in Section 3.3.1 and 3.4.
		Section 5.2: • Clarified analysis method for unadjusted GMR.
		Section 6.1.2: • The propensity score method was replaced with random selection with matching factors sex and age (in years) to ensure comparable distribution in each cohort.
		Section 6.1.2.1, 6.1.2.2, 6.1.2.5, and 6.1.2.6 • Added sensitivity immunogenicity analyses for unadjusted GMR and difference of SR using Immunogenicity Analysis Per Protocol Set, mITT, and changed adjusted sensitivity analysis using Immunogenicity Analysis Set.
		Section 6.4 and 6.5: • Diabetes was deleted from demographic table and subgroup analysis. • Age group 18-55 vs 56-85 years was added for subgroup analysis.
		Section 6.6.1 • Summary of lab safety assessment at screening was changed to be based on All Randomized Subjects.
4/30 Jan 2024	Protocol V7.0, 28 Jun 2023	Section 3.1.1.1 Updated the calculation of duration of reactogenicity event to "Duration will be calculated in days as the difference from the start of the first reported event to the resolution of the last reported event, inclusive. The resolution date for events lasting longer than 7 days was recorded in the subject's adverse event case report form. If the resolution date is partial or missing, the duration will be considered unknown."

Version/ date	Associated protocol	Summary and rationale for changes
		Section 3.3.1 Updated definition of confirmed COVID-19 cases.
		Section 3.3 Added exploratory endpoint CD4+ and CD8+ T-cell responses (Ex vivo ELISpot) for Part A with endpoint description in Section 3.3.3 and analysis in Section 6.3.1.2.
		Section 3.4.1 Updated Definition of baseline value and SARS-CoV-2 infection at baseline and post vaccination
		Section 4 Added the clarification regarding how to handle mis-dosed subjects into safety and immunogenicity analysis sets.
		Section 6.1.1 Added "The summary analyses post dose 2 and post dose 3 of IMP will be performed with subjects who received two doses and three doses of IMP to calculate the percentages".
		Section 6.1.1.1 Added 6.1.1.1 Safety Overview.
		Section 6.1.1.4, 6.1.1.5 Added number of events in the AE and SAE summary tables.
		Section 6.1.2.1-4, 6.1.3.1-2 Remove sensitivity analyses using the Immunogenicity Analysis Per Protocol Set and All-available Immunogenicity Set (mITT).
		Section 6.1.2.7 and Section 6.1.2.8 These two analyses will not be performed.
		Section 6.3.1.1 Added CD4+ and CD8+ T-cell responses (Ex vivo ELISpot).
		Section 6.4 and 6.5 Resume diabetes (yes and no) as subgroup factor in Part B and C.
		Section 7.1 Updated based on the actual analysis conducted.
		Section 8.1, 8.2 and 8.3 Updated Section 8.1 Additional analysis sets for immunogenicity analysis. Added Section 8.2 Changes in interim analysis Added Section 8.3 Withdrawal of primary objectives to demonstrate the non-inferiority of

Version/ date	Associated protocol	Summary and rationale for changes
		immune response against VOC (B.1.1.7 and B.1.617.2) after two doses of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in terms of GMT and SR for COVID-19 vaccine-naïve subjects in Part B Cohort 6.
		Appendix 2 Added Appendix 2. Subject selection for primary and secondary immunogenicity analysis in Part B
5/23 Feb 2024	Protocol V7.0, 28 Jun 2023	Remove "At the time this risk assessment was authored" from Section 8.3.

2 INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in trial BNT162-17 (Protocol Version 7.0; dated 28Jun2023). Any modifications of the statistical analysis outlined in the protocol are documented in Section 8.

2.1 Trial objectives, estimands, and endpoints

The estimands and endpoints corresponding to each primary, secondary, and exploratory objective are described in Table 2, Table 3, and Table 4 below.

Table 2. List of objectives, estimands, and endpoints for Part A

OBJECTIVES	ESTIMANDS	ENDPOINTS
Primary objectives (Safety)		
To describe the safety profile of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), given as one or two booster doses to BNT162b2-experienced subjects, or as three doses to COVID-19 vaccine-naïve subjects. To describe the safety profile of the monovalent vaccine BNT162b2 (B.1.1.7) given as one booster dose to BNT162b2-experienced subjects. * To describe the safety profile of the monovalent vaccine BNT162b2 (B.1.617.2), given as one booster dose to BNT162b2-experienced subjects. * To describe the safety profile of BNT162b2, given as one booster dose to BNT162b2-experienced subjects. *	For each trial treatment, in subjects receiving at least one dose of IMP, the percentage of subjects reporting: • Local reactions at the injection site up to 7 days after each dose. • Systemic events up to 7 days after each dose. • AEs from Dose 1 up to 1 month after each dose. • SAEs from Dose 1 up to 6 months after the last dose.	• Local reactions (pain, tenderness, erythema/redness, induration/swelling) • Systemic events (fever, fatigue, headache, chills, vomiting, nausea, diarrhea, new or worsened muscle pain, and new or worsened joint pain) • AEs • SAEs
Secondary objectives (Immunogenicity)		
To describe the immune response after one, two, or three doses of BNT162b2 (B.1.1.7 + B.1.617.2), BNT162b2 (B.1.1.7), BNT162b2 (B.1.617.2), and BNT162b2.	For BNT162b2-experienced subjects: • GMTs at each time point. • GMFR from before vaccination to each subsequent time point after vaccination. • Seroresponse (SR) in terms of NT at each postvaccination time point.	Reference and VOC specific NTs
Exploratory objectives		
To comprehensively describe B-cell responses after one, two, or three doses of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), or	For a subset of subjects in Part A: • Characterization of SARS-CoV-2 S protein-specific B cells to	• Frequency and phenotypic characterization of SARS-CoV-2 spike-specific B cells

OBJECTIVES	ESTIMANDS	ENDPOINTS
one booster dose of the monovalent vaccine BNT162b2 (B.1.1.7), or one booster dose of BNT162b2 (B.1.617.2), at 30 µg.	identify B cells recognizing conserved and strain-specific epitopes.	
To describe the T cell-mediated immune response after one, two, or three doses of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), or one booster dose of the monovalent vaccine BNT162b2 (B.1.1.7), or one booster dose of BNT162b2 (B.1.617.2), at 30 µg.	For a subset of subjects in Part A: • CMI responses including CD4 and CD8 T-cell responses to S and RBD antigens of the reference strain and the B.1.1.7 and B.1.617.2 VOC.	• Reference and VOC specific CD4 and CD8 T-cell responses (e.g., using ELISpot, ICS)
To evaluate the immune response over time to prophylactic multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) and persistence of immune response in subjects with and without prior COVID-19 vaccination.	• GMC/GMT and GMFR at baseline and 1, 6, and 12 months after completion of vaccination. • Seroresponse for reference strain and B.1.1.7 and B.1.617.2 variant strains.	• Reference and VOC specific NTs • RBD and full length S-binding or S1-binding Ig levels
To evaluate SARS-CoV-2 viral sequences in subjects.	• SARS-CoV-2 S antigen sequences or whole viral genome sequencing of interest.	• Viral sequences
To evaluate cross-neutralization of vaccine-induced antibodies to emerging SARS-CoV-2 variants.	• For a subset of subjects, after any dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) or monovalent vaccine BNT162b2, BNT162b2 (B.1.1.7) and BNT162b2 (B.1.617.2) vaccines, measured cross-neutralization of other SARS-CoV-2 variants (e.g., using VNT or pVNT).	• NT data

* Note: "BNT162b2-experienced" is defined as subjects who have previously received two injections of 30 µg BNT162b2.
Abbreviations: AE = adverse event; CD = cluster of differentiation (e.g., CD4, CD8); CMI = cell-mediated immunity; ELISpot = enzyme-linked immunospot; GMC = geometric mean concentration; GMFR = geometric mean fold rises; GMT = geometric mean titer; ICS = intracellular cytokine staining; Ig = immunoglobulin; NT = neutralizing titers; pVNT = pseudo-virus neutralization test; RBD = receptor binding domain; SAE = serious adverse event; SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2; S = spike protein; SR = seroresponse; VNT = virus neutralization test; VOC = variant(s) of concern.

Table 3. List of objectives, estimands, and endpoints for Part B

OBJECTIVES	ESTIMANDS	ENDPOINTS
Primary objectives (Safety)		
To describe the safety profile of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) given as one booster dose to BNT162b2-experienced subjects *, or as three doses to COVID-19 vaccine-naïve subjects. To describe the safety profile of the monovalent vaccine	In subjects receiving at least one dose of IMP, the percentage of subjects reporting: • Local reactions at the injection site up to 7 days after each dose. • Systemic events up to 7 days after each dose.	• Local reactions (pain, tenderness, erythema/redness, induration/swelling) • Systemic events (fever, fatigue, headache, chills, vomiting, nausea, diarrhea, new or worsened muscle

OBJECTIVES	ESTIMANDS	ENDPOINTS
BNT162b2 (B.1.617.2) given as one booster dose to BNT162b2-experienced subjects *.	• AEs from Dose 1 up to 1 month after each dose. • SAEs from Dose 1 up to 6 months after the last dose.	pain, and new or worsened joint pain) • AEs • SAEs
Primary objectives (Immunogenicity)		
BNT162b2-experienced subjects		
To demonstrate the non-inferiority of immune response against VOC (B.1.1.7 and B.1.617.2) after one dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in terms of GMT.	• GMR of B.1.1.7 NT 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2. • GMR of B.1.617.2 NT 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2.	• Reference and VOC specific NTs
To demonstrate the non-inferiority of immune response against VOC (B.1.617.2) after one dose of monovalent vaccine BNT162b2 (B.1.617.2) in terms of GMT.	• GMR of B.1.617.2 NT 1 month after one dose of BNT162b2 (B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2.	• Reference and VOC specific NTs
To demonstrate the non-inferiority of immune response against VOC (B.1.1.7 and B.1.617.2) after one dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in terms of SR.	• The difference in SRs to B.1.1.7 NT 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2. • The difference in SRs to B.1.617.2 NT 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2.	• Reference and VOC specific SRs
Primary objectives (Immunogenicity)		
BNT162b2-experienced subjects		
To demonstrate the non-inferiority of immune response against VOC (B.1.617.2) after one dose of monovalent vaccine BNT162b2 (B.1.617.2) in terms of SR.	• The difference in SRs to B.1.617.2 NT 1 month after one dose of BNT162b2 (B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2.	• Reference and VOC specific SRs
COVID-19 vaccine-naïve subjects		
To demonstrate the non-inferiority of immune response against reference strain after one dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects with evidence of prior infection to the immune response after	• GMR of reference strain NT 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects with evidence of prior infection to the reference strain NT 1 month after two	• Reference strain NTs

OBJECTIVES	ESTIMANDS	ENDPOINTS
two doses of original BNT162b2 in subjects without evidence of infection from the Phase III trial BNT16202 / C4591001 in terms of GMT.	doses of BNT162b2 in subjects without evidence of infection.	
To demonstrate the non-inferiority of immune response against reference strain after one dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects with evidence of prior infection to the immune response after two doses of original BNT162b2 in subjects without evidence of infection from the Phase III trial BNT16202 / C4591001 in terms of SR.	• The difference in SRs to the reference strain NT 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects with evidence of prior infection and to the reference strain NT 1 month after two doses of BNT162b2 in subjects without evidence of infection.	• Reference strain NTs
To demonstrate the non-inferiority of immune response against VOC (B.1.1.7 and B.1.617.2) after two doses of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in terms of GMT.	• GMR of B.1.1.7 NT 1 month after two doses of BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2. • GMR of B.1.617.2 NT 1 month after two doses of BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2.	• Reference and VOC specific NTs
To demonstrate the non-inferiority of immune response against VOC (B.1.1.7 and B.1.617.2) after two doses of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in terms of SR.	• The difference in SRs to B.1.1.7 NT 1 month after two doses of BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2. • The difference in SRs to B.1.617.2 NT 1 month after two doses of BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT 1 month after two doses of BNT162b2.	• Reference and VOC specific SRs
Secondary objectives		
BNT162b2-experienced subjects		
To describe the immune response against the reference strain and VOCs after one dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) vs two doses of BNT162b2.	• GMTs and SRs of VOCs and reference strain NT 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) and Dose 2 of BNT162b2.	• Reference and VOC specific NTs
To describe the immune response against the reference strain and VOCs after one dose of monovalent vaccine BNT162b2 (B.1.617.2) vs two doses of BNT162b2.	• GMTs and SRs of VOCs and reference strain NT 1 month after one dose of BNT162b2 (B.1.617.2) and Dose 2 of BNT162b2.	• Reference and VOC specific NTs
COVID-19 vaccine-naïve subjects		

OBJECTIVES	ESTIMANDS	ENDPOINTS
To describe the immune response against the reference strain and VOCs after three doses of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2).	• GMTs and SRs of VOCs and reference strain NT 1 month after Dose 2 and Dose 3 of BNT162b2 (B.1.1.7 + B.1.617.2).	• Reference and VOC specific NTs
To descriptively compare the immune response against VOCs 3 weeks after one dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects with evidence of prior infection to the immune response 1 month after one booster dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in BNT162b2-experienced subjects without evidence of prior infection.	• GMTs and SRs of VOCs NT 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects with evidence of prior infection and 1 month after one booster dose of BNT162b2 (B.1.1.7 + B.1.617.2) in BNT162b2-experienced subjects without evidence of prior infection. • GMR of VOCs NT 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects with evidence of prior infection to the VOCs NT 1 month after one booster dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects without evidence of prior infection. • The difference in SRs to VOCs NT 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects with evidence of prior infection and to the VOCs NT 1 month after one booster dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects without evidence of prior infection.	• VOC specific NTs (B.1.1.7, B.1.617.2, B.1.1.529.5 [Omicron BA.5])
To descriptively compare the immune response against VOCs 3 weeks after one dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects with evidence of prior infection to the immune response 1 month after two doses of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects without evidence of prior infection.	• GMTs and SRs of VOCs NT 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects with evidence of prior infection and 1 month after two doses of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects without evidence of prior infection. • GMR of VOCs NT 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects with evidence of prior infection to the VOCs NT 1 month after two doses of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects without evidence of prior infection. • The difference in SRs to VOCs NT 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects with evidence of prior	• VOC specific NTs (B.1.1.7, B.1.617.2, B.1.1.529.5 [Omicron BA.5])

OBJECTIVES	ESTIMANDS	ENDPOINTS
	infection and to the VOCs NT 1 month after two doses of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects without evidence of prior infection.	
Exploratory objectives		
To evaluate SARS-CoV-2 viral sequences in subjects.	• SARS-CoV-2 S antigen sequences or whole viral genome sequencing of interest.	• Viral sequences
To evaluate the immune response over time to prophylactic multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) or monovalent vaccine BNT162b2 (B.1.617.2), and persistence of immune response in subjects with and without prior COVID-19 vaccination.	• GMC/GMT and GMFR at baseline and 1, 6, and 12 months after completion of vaccination. • SRs for reference strain and B.1.1.7 and B.1.617.2 variant strains.	• Reference and VOC specific NTs • RBD and full-length S-binding or S1-binding Ig levels
To evaluate cross-neutralization of vaccine-induced antibodies to emerging SARS-CoV-2 variants.	• For a subset of subjects, after any dose of multivalent vaccine, BNT162b2 (B.1.1.7 + B.1.617.2) or monovalent vaccines BNT162b2 and BNT162b2 (B.1.617.2) vaccines, measured cross-neutralization of other SARS-CoV-2 variants (e.g., using VNT or pVNT).	• NT data
To describe B-cell responses after one or two and three doses of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2).	For a subset of Cohort 6 subjects in Part B: • Characterization of SARS-CoV-2 S protein-specific B cells to identify B cells recognizing conserved and strain-specific epitopes.	• Frequency and phenotypic characterization of SARS-CoV-2 spike-specific B cells
To describe the T cell-mediated immune response after one or two and three doses of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2).	For a subset of Cohort 6 subjects in Part B: • CMI responses including CD4 and CD8 T-cell responses to S and RBD antigens of the reference strain and the B.1.1.7 and B.1.617.2 variant strains.	• Reference and VOC specific CD4 and CD8 T-cell responses (e.g., using ELISpot, ICS)
To describe the incidence of non-S seroconversion to SARS-CoV-2 in subjects with and without prior COVID-19 vaccination who received prophylactic multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) or monovalent vaccine BNT162b2 (B.1.617.2).	• Incidence of non-S-seroconversion to SARS-CoV-2 per 1,000 person-years of follow-up.	• Nucleocapsid (N)-binding antibody seroconversion in subjects with no serological or virological evidence of past SARS-CoV-2 infection or confirmed COVID-19
To describe the incidence of confirmed COVID-19 at 1 year follow-up period in subjects with and without prior COVID-19 vaccination who received prophylactic multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) or	• Incidence of confirmed COVID-19 cases per 1,000 person-years of follow-up.	• Number of confirmed COVID-19 cases

OBJECTIVES	ESTIMANDS	ENDPOINTS
monovalent vaccine BNT162b2 (B.1.617.2).		

* Note: "BNT162b2-experienced" is defined as subjects who have previously received two injections of 30 µg BNT162b2.
Abbreviations: AE = adverse event; CD = cluster of differentiation (e.g., CD4, CD8); CMI = cell-mediated immunity; ELISpot = enzyme-linked immunospot; GMC = geometric mean concentration; GMFR = geometric mean fold rises; GMT = geometric mean titer; GMR = geometric mean ratio; ICS = intracellular cytokine staining; Ig = immunoglobulin; NT = neutralizing titers; pVNT = pseudo-virus neutralization test; SAE = serious adverse event; SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2; SR = seroresponse; VNT = virus neutralization test; VOC = variant(s) of concern.

Table 4. List of objectives, estimands, and endpoints for Part C

OBJECTIVES	ESTIMANDS	ENDPOINTS
Primary objectives (Safety)		
To describe the safety profile of the monovalent vaccine BNT162b2 (B.1.1.529) given as one dose to RNA-based COVID-19 vaccine-experienced subjects with history of SARS-CoV-2 infection.	In subjects receiving at least one dose of IMP, the percentage of subjects reporting: • Local reactions at the injection site up to 7 days after each dose. • Systemic events up to 7 days after each dose. • AEs from Dose 1 up to 1 month after the last dose. • SAEs from Dose 1 up to 6 months after the last dose.	• Local reactions (pain, tenderness, erythema/redness, induration/swelling) • Systemic events (fever, fatigue, headache, chills, vomiting, nausea, diarrhea, new or worsened muscle pain, and new or worsened joint pain) • AEs • SAEs
To describe the safety profile of the monovalent vaccine BNT162b2 given as one dose to RNA-based COVID-19 vaccine-experienced subjects with history of SARS-CoV-2 infection.	In subjects receiving at least one dose of IMP, the percentage of subjects reporting: • Local reactions at the injection site up to 7 days after each dose. • Systemic events up to 7 days after each dose. • AEs from Dose 1 up to 1 month after the last dose. • SAEs from Dose 1 up to 6 months after the last dose.	• Local reactions (pain, tenderness, erythema/redness, induration/swelling) • Systemic events (fever, fatigue, headache, chills, vomiting, nausea, diarrhea, new or worsened muscle pain, and new or worsened joint pain) • AEs • SAEs
Primary objectives (Immunogenicity)		
To describe the humoral immune response against SARS-CoV-2 variants after one dose of BNT162b2 (B.1.1.529) or after one dose of BNT162b2 in RNA-based COVID-19 vaccine-experienced subjects with history of SARS-CoV-2 infection.	• GMR and difference in SR of B.1.1.529 NT 1 month after one dose of BNT162b2 (B.1.1.529) to those at 1 month after one dose of BNT162b2 for Cohorts 7 and 8.	• VOC specific NTs • VOC specific SRs
Secondary objectives		

OBJECTIVES	ESTIMANDS	ENDPOINTS
To describe the humoral immune response against SARS-CoV-2 variants after one dose of BNT162b2 (B.1.1.529) or after one dose of BNT162b2 or a post SARS-CoV-2 infection in RNA-based COVID-19 vaccine-experienced subjects with history of SARS-CoV-2 infection.	• GMT of VOC NT at baseline and 7 days, 1 month, and 3 months after the trial start for Cohorts 7, 8, and 9, and 6 and 12 months after the trial start for Cohorts 7 and 8.	• VOC specific NTs
Exploratory objectives		
To evaluate SARS-CoV-2 viral sequences in subjects.	• SARS-CoV-2 S antigen sequences or whole viral genome sequencing of interest.	• Viral sequences
To evaluate cross-neutralization of vaccine-induced antibodies to ancestral and emerging SARS-CoV-2 variants.	• For a subset of subjects, after one dose of monovalent vaccine BNT162b2 (B.1.1.529) or BNT162b2, measured cross-neutralization of other SARS-CoV-2 variants (e.g., using VNT or pVNT).	• NT data
To evaluate the immune response over time to prophylactic monovalent vaccine BNT162b2 (B.1.1.529) or BNT162b2 and persistence of immune response in RNA-based COVID-19 vaccine-experienced subjects with history of SARS-CoV-2 infection.	• GMC/GMT and GMFR at baseline, 7 days, and 1, 3, 6, and 12 months after completion of vaccination. • Seroresponse for B.1.1.529 variant strain.	• Reference and VOC specific NTs • RBD and full length S-binding or S1-binding Ig levels
To evaluate the immune response to SARS-CoV-2 in subjects.	• GMC/GMT and GMFR at baseline, 7 days, and 1, 3, 6, and 12 months after completion of vaccination.	• Nucleocapsid (N)-binding Ig levels
To describe the incidence of confirmed COVID-19 at 1 year follow-up period in subjects who received one dose of monovalent BNT162b2 (B.1.1.529) or BNT162b2.	• Incidence of confirmed COVID-19 cases per 1,000 person-years of follow-up.	• Number of confirmed COVID-19 cases
To describe the B cell- and T cell-mediated immune response to monovalent BNT162b2 (B.1.1.529) and to BNT162b2 and persistence of immune response in subjects.	For a subset of subjects in Part C: • Characterization of SARS-CoV-2 S protein-specific B cells to identify B cells recognizing conserved and strain-specific epitopes. • CMI responses including CD4 and CD8 T-cell responses to S and RBD antigens of the reference strain and B.1.1.529 variant strain.	• Frequency and phenotypic characterization of SARS-CoV-2 spike-specific B cells • Reference and VOC specific CD4 and CD8 T-cell responses (e.g., using ELISpot, ICS)

Note: BNT162b2 (B.1.1.529) refers to the monovalent vaccine specific for SARS-CoV-2 Omicron subvariant BA.1, i.e., B.1.1.529.1.
Abbreviations: AE = adverse event; CD = cluster of differentiation (e.g., CD4, CD8); CMI = cell-mediated immunity; ELISpot = enzyme-linked immunospot; GMC = geometric mean concentration; GMFR = geometric mean fold rises; GMT = geometric mean titer; GMR = geometric mean ratio; ICS = intracellular cytokine staining; Ig = immunoglobulin; NT = neutralizing titers; pVNT = pseudo-virus neutralization test; RBD = receptor binding domain; S = spike protein; SAE = serious adverse event; SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2; SR = seroresponse; VNT = virus neutralization test; VOC = variant(s) of concern.

2.2 Trial design

2.2.1 Overall design

This is a Phase II open-label trial.

This trial consists of three parts, Part A, Part B, and Part C, and will evaluate the safety and immunogenicity of a third booster injection of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), and the safety and immunogenicity of a third booster injection of the monovalent vaccine BNT162b2 (B.1.617.2) or BNT162b2 (B.1.1.7), in subjects who have received two doses of the parent vaccine BNT162b2 at 30 µg, at least 6 months after the second dose of BNT162b2. It will also evaluate the safety and immunogenicity of a three-dose regimen of BNT162b2 (B.1.1.7 + B.1.617.2) in subjects who have not received prior COVID-19 vaccination. In addition, the safety and immunogenicity of BNT162b2 (B.1.1.529) or BNT162b2 given as a third or fourth vaccine dose to RNA COVID-19 vaccine-experienced subjects with history of SARS-CoV-2 infection will be evaluated and contrasted with the natural immune response reached after infection with the SARS-CoV-2 Omicron variant.

Part A will describe, in parallel subject cohorts, the safety and the immunogenicity of BNT162b2 (B.1.1.7 + B.1.617.2) in relation to the corresponding monovalent vaccines BNT162b2 (B.1.1.7) and BNT162b2 (B.1.617.2), and the parent vaccine BNT162b2. Comprehensive assessments of the humoral and cell-mediated immune responses in those groups will evaluate if the immunological responses to each antigen is undeterred by the addition of additional antigens in BNT162b2 (B.1.1.7 + B.1.617.2). Further, analyses of the B cell compartment, including memory B cells, will assess if BNT162b2 (B.1.1.7 + B.1.617.2) as a booster in prior vaccinated subjects elicits a qualitatively improved anamnestic response, with expansion of a diversified memory B cell population able to cross-neutralize conserved and unique strain epitopes. Part A will provide rapid information about the safety and the immunogenicity of the multivalent BNT162b2 (B.1.1.7 + B.1.617.2) vaccine in a previously vaccinated group and in a vaccine-naïve population, and will inform Part B in terms of dosage and sample size.

Part B will be initiated after review of reactogenicity and available immunogenicity data of Part A (Cohorts 1, 4, 6) by the SRC. Part B will compare the immune response after one dose or after two doses of the multivalent BNT162b2 (B.1.1.7 + B.1.617.2) vaccine against the variant strains or after one dose of multivalent BNT162b2 (B.1.1.7 + B.1.617.2) vaccine against the reference ancestral SARS-CoV-2 strain [Wuhan-Hu-1/ USA-WA1] vs the immune response after two doses of BNT162b2 against the reference strain observed in subjects from the Phase III BNT162-02 / C4591001 (NCT04368728) trial. Part B will also compare the immune response after one dose of the monovalent vaccine BNT162b2 (B.1.617.2) against the variant strain vs the immune response after two doses of BNT162b2 against the reference strain observed in subjects from the Phase III BNT162-02 / C4591001 trial. Part B will further include the same assessments of the B cell and T cell compartments as described in Part A in a pre-defined sample size of the cohort population.

Based on data from Part A, the dosage, sample size and trial groups planned in Part B may be adjusted via a protocol amendment.

Part C will include healthy subjects who were previously vaccinated with two or three doses of any authorized COVID-19 RNA-based vaccine and were subsequently diagnosed with a SARS-CoV-2 infection from January 2022 onwards (and limited to a period when there was a high prevalence of SARS-CoV-2 Omicron infections). Part C will compare immune responses against the SARS-CoV-2 Omicron variant after one dose of 30 µg monovalent BNT162b2 (B.1.1.529) vaccine vs the immune response after one dose of 30 µg BNT162b2. In addition, Part C (Cohort 9) will evaluate the immune response reached after infection with the SARS-CoV-2 Omicron variant.

Part A

Part A consists of six parallel cohorts of approximately n = 20 subjects each, which will enroll subjects 18 to 55 years of age (Cohorts 1 to 6).

Cohorts 1 to 5 will enroll subjects who received two injections of 30 µg BNT162b2, at least 6 months after the second BNT162b2 dose in the following five cohorts:

- **Cohort 1**: Subjects will receive one dose of 30 µg multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) on Day 1 (baseline in this trial), consisting of a 1:1 mixture of two BNT162b2 monovalent vaccines: 15 µg BNT162b2 (B.1.1.7), and 15 µg BNT162b2 (B.1.617.2).
- **Cohort 2**: Subjects will receive two doses of 30 µg multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), one each on Day 1 and on Day 56 (8 weeks apart).
- **Cohort 3**: Subjects will receive one dose of 30 µg monovalent vaccine BNT162b2 (B.1.1.7) on Day 1.
- **Cohort 4**: Subjects will receive one dose of 30 µg monovalent vaccine BNT162b2 (B.1.617.2) on Day 1.
- **Cohort 5**: Subjects will receive one dose of 30 µg BNT162b2 on Day 1.

Cohort 6 will enroll subjects who have not received any prior prophylactic vaccine against COVID-19.

- **Cohort 6**: Subjects will receive three doses of 30 µg multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), one each on Day 1 and Day 21 and the third dose ~6 months after the second dose.

Part A will comprehensively describe, through the parallel Cohorts 1 to 5, the safety and the immunogenicity of one or two doses of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), in relation to the corresponding monovalent vaccine BNT162b2 (B.1.1.7) and BNT162b2 (B.1.617.2) vaccines, and the parent vaccine BNT162b2. A total dose of 30 µg is selected for the BNT162b2-based vaccines against variant strains to be tested in this trial, based on preliminary safety results of the monovalent vaccine BNT162b2 (B.1.351) vaccine against the SARS-CoV-2 B.1.351 variant strain, currently being tested as a third booster dose in the BNT162-02 / C4591001 trial. For Cohorts 1 to 4, an optional additional lower dose level may be tested in up to 20 additional subjects per cohort, based on safety and immunogenicity data obtained with the 30 µg dosage level.

Comprehensive assessments of the humoral and cell-mediated immune responses in Cohorts 1 to 5 will evaluate if the immunological responses to each VOC is unaffected by the addition of additional antigens in the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2). Further, analyses of the B cell compartment, including memory B cells, will assess if the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) as a booster in prior vaccinated subjects elicits a qualitatively improved anamnestic response, with expansion of a diversified memory B cell population able to cross-neutralize conserved and unique strain epitopes.

Twenty (20) subjects who are COVID-19 vaccine-naïve and have not experienced COVID-19, will be enrolled in Cohort 6 to receive BNT162b2 (B.1.1.7 + B.1.617.2) given as a three-dose regimen – the first and second doses administered on Day 1 and Day 21 and the third dose ~6 months after the second dose.

Part B

Part B will be initiated after review of reactogenicity and available immunogenicity data of Part A (Cohorts 1, 4, 6) by the SRC.

Part B consists of three cohorts of n = 300 subjects each (~375 subjects will be enrolled in each cohort to ensure there are 300 evaluable subjects), which will enroll subjects 18 to 85 years old; ~60% of these subjects should be 18 to 55 years old and ~40% should be 56 to 85 years old:

- **Cohort 1** will enroll subjects from the trial BNT162-02 / C4591001 who received two injections of 30 µg BNT162b2, at least 6 months after the second BNT162b2 dose. Subjects will receive on Day 1 one dose of 30 µg multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2).
- **Cohort 4** will enroll subjects from the trial BNT162-02 / C4591001 who received two injections of 30 µg BNT162b2, at least 6 months after the second BNT162b2 dose. Subjects will receive on Day 1 one dose of 30 µg monovalent vaccine BNT162b2 (B.1.617.2).
- **Cohort 6** will enroll subjects who have not received any prior prophylactic vaccine against COVID-19. Subjects will receive 30 µg multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), one each on Day 1 and Day 21 and the third dose ~6 months after the second dose. Approximately 15 subjects at preselected sites in Part B will be evaluated in terms of cell-mediated immunity.

Part B will expand the safety information of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) as one booster dose in BNT162b2-experienced subjects, and as two-dose, primary vaccination regimen in subjects who are COVID-19 vaccine-naïve and have not experienced COVID-19.

Part B will also determine based on geometric mean ratios (GMR) of neutralizing titers and seroresponse rate, if the immune response of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) against the B.1.1.7 and the B.1.617.2 strains or immune response after one dose of multivalent BNT162b2 (B.1.1.7 + B.1.617.2) vaccine against the reference strain, and the immune response of the monovalent vaccine BNT162b2 (B.1.617.2) against the B.1.617.2 strain, is immunobridged to the immune response observed against the

reference strain in selected existing subjects from the Phase III trial BNT162-02 / C4591001, who received two doses of BNT162b2. The selection will ensure comparable distribution of age and sex in the control group and the BNT162b2 (B.1.1.7 + B.1.617.2) and BNT162b2 (B.1.617.2) groups. To achieve 300 evaluable subjects for each of the immunobridging comparisons, 20% non-evaluable rate is estimated, which results in the enrollment of 375 subjects in each of Part B cohorts.

In addition, Part B will compare the immune response against the reference and the variant strains after one or two doses of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), compared to after two doses of BNT162b2 in subjects from the Phase III BNT162-02 / C4591001 trial.

Part C

Part C consists of n = ~225 subjects who previously received two or three injections of any authorized COVID-19 RNA-based vaccine and were subsequently diagnosed with SARS-CoV-2 infection from January 2022 onwards (limited to a period when there was a high prevalence of SARS-CoV-2 Omicron infections).

Subjects in Part C will be randomized in a 2:2:1 ratio into three cohorts; ~90 subjects each in Cohorts 7 and 8 (to ensure ~80 evaluable subjects in each cohort after 11% dropout) and 45 subjects in Cohort 9. Randomization will be stratified by age group (18 to 55 years of age or 56 to 85 years of age) and by number of prior doses of COVID-19 RNA-based vaccine (two or three doses).

The sponsor may terminate the enrollment into Cohort 9 at any time in case the evolving COVID-19 epidemiology and/or the recommendations issued in the context of national vaccination campaigns no longer support enrollment of the trial population in a realistically feasible time.

Cohorts 7 to 9 will enroll subjects 18 to 85 years old; ~60% of these subjects should be 18 to 55 years old and ~40% should be 56 to 85 years old:

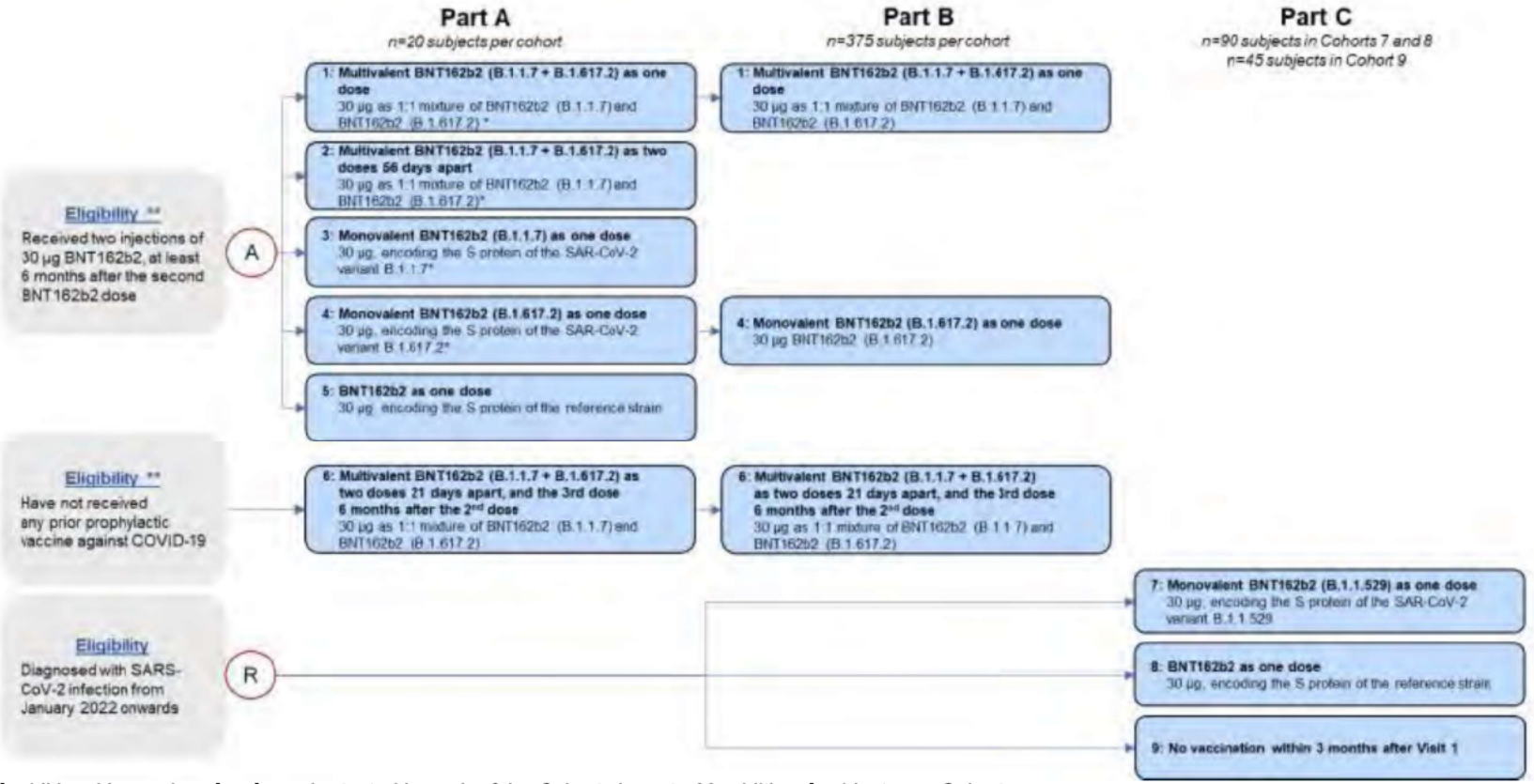
- **Cohort 7**: Subjects will receive one dose of 30 µg monovalent vaccine BNT162b2 (B.1.1.529) on Day 1.
- **Cohort 8**: Subjects will receive one dose of 30 µg BNT162b2 on Day 1.
- **Cohort 9**: No vaccination will be given to Cohort 9 subjects within 3 months after Visit 1. After the 3-month follow-up period, subjects in Cohort 9 will be offered a BNT162b2 vaccination, depending on the epidemiological situation, local regulatory authority recommendations, and/or variant vaccine authorization status.

Approximately 25 subjects at preselected sites in each cohort of Part C will be evaluated in terms of cell-mediated immunity.

2.2.2 Trial schema

A summary of the trial as a flow diagram is displayed in Figure 1. For the planned assessments and visits, see the Schedule of Activities (SoA) in Section 1.3 of the trial protocol (version 7.0; 28Jun2023).

Figure 1. Schema – Safety and immunogenicity of SARS-CoV-2 monovalent and multivalent RNA-based vaccines in healthy subjects



* An optional additional lower dose level may be tested in each of the Cohorts in up to 20 additional subjects per Cohort.
** No history of COVID-19 and/or clinical or evidence of prior infection with SARS-CoV-2 at screening (Visit 0).
Abbreviations: "A" = assignment to Cohort; "R" = randomization.

3 ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1 Primary endpoints

The primary objective for Part A is solely based on safety endpoints, however Part B and Part C also have immunogenicity endpoints that are also primary objectives.

3.1.1 Safety endpoints for Parts A, B, and C

For subjects in each cohort, in Part A, Part B and Part C of this trial, who received at least 1 dose of trial IMP (Investigational Medicinal Product), the primary safety endpoints are:

- Local reactions up to 7 days after each dose.
- Systemic events up to 7 days after each dose.
- Adverse events (AEs) from:
  - Dose 1 up to 1 month after Dose 1
  - Dose 2 up to 1 month after Dose 2
  - Dose 3 up to 1 month after Dose 3
- Serious adverse events (SAEs) from Dose 1 up to 6 months after the last dose.

For Cohorts 1, 3, 4, 5, 7, and 8, last dose is Dose 1. Dose 2 is the last dose for subjects in Cohort 2 and Dose 3 is the last dose for subjects in Cohort 6. Cohort 9 participants are not dosed therefore their safety data will not contribute to the primary safety endpoint.

3.1.1.1 Local reactions

The local reactions assessed by trial subjects and reported in the e-diary are pain at the injection site, tenderness, erythema/redness, induration/swelling, from Day 1 through Day 7 after each trial IMP dose, where Day 1 is the day of each IMP injection. A subject is deemed to have had a local reaction if they report the reaction as "yes" on any day of the e-diary recording period (i.e., Day 1 through Day 7 inclusive).

For redness and swelling, the reported size must be at least 2.5 cm (0.98 inches) to be deemed as a local reaction. Redness and swelling with a size less than 2.5 cm will not be included in the analysis.

Solicited local reactions will be categorized as mild, moderate, severe, or potentially life-threatening) and graded from 1 to 4, during the analysis as described in Table 5. The categories and grades are based on the FDA Guidance for Industry: "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials".

Table 5. Local reaction grading scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Pain at the injection site	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room visit or hospitalization for severe pain
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room visit or hospitalization
Erythema /redness [a]	2.5 cm to 5.0 cm (0.98 in to 1.96 in)	>5.0 cm to 10.0 cm (>1.96 in to 3.94 in)	>10 cm (>3.94 in)	Necrosis or exfoliative dermatitis
Induration /swelling [b]	2.5 cm to 5.0 cm (0.98 in to 1.96 in)	>5.0 cm to 10.0 cm (>1.96 in to 3.94 in)	>10 cm (>3.94 in)	Necrosis

a) In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

b) Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

For subjects recording at least one local reaction from Day 1 through Day 7 after IMP injection:

- The maximum severity grade is equal to the highest graded local reaction within the recording period.
- Duration will be calculated in days as the difference from the start of the first reported event to the resolution of the last reported event, inclusive. The resolution date for events lasting longer than 7 days was recorded in the subject's adverse event case report form. If the resolution date is partial or missing, the duration will be considered unknown.
- Onset day, defined as the first day of reporting any severity, will be derived for each recorded local reaction. If a subject report changes in severity of the local reaction, only the first day of reporting that specific local reaction will be counted. Local reaction onset day for Dose 1 will be calculated from the IMP Dose 1 injection date, for Dose 2 from the IMP Dose 2 date and for Dose 3 from the IMP Dose 3 date.

3.1.1.2 Systemic events

Symptoms of systemic events assessed by trial subjects and recorded in the e-diary are fever, fatigue, headache, chills, vomiting, nausea, diarrhea, new or worsened muscle pain, and new or worsening joint pain from Day 1 through Day 7 inclusive, where Day 1 is the day of each IMP injection. The derivations for systemic events will be handled similar to the way local reactions are handled for presence of an event, severity level, duration, and onset day.

Fever is defined as an oral temperature ≥38.0°C (100.4°F). Temperatures collected in degrees Fahrenheit will be converted to degrees Celsius using (°F – 32) × 5/9 and grading will be based on the unrounded conversions. An investigator or medically qualified person

must confirm a trial subject's fever as >40.0°C/>104.0°F before it is entered in the trial database.

The highest oral temperature for each day will be recorded in the e-diary and the maximum temperature from Day 1 through Day 7 will be graded as described in Table 6.

Solicited systemic events will be categorized as mild, moderate, severe, or potentially life-threatening) and graded from 1 to 4, during the analysis as described in Table 6.

Table 6. Systemic events grading scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Fever (oral temperature of ≥38.0°C/≥100.4°F)	≥38.0°C/≥100.4°F to 38.4°C/101.1°F	≥38.5°C/≥101.2°F to 38.9°C/102.0°F	≥39.0°C/≥102.1°F to 40.0°C/104.0°F	>40.0°C/>104.0°F
Vomiting	1 to 2 times in 24 h	>2 times in 24 h	Requires intravenous hydration	Emergency room visit or hospitalization for hypotensive shock
Diarrhea	2 to 3 loose stools in 24 h	4 to 5 loose stools in 24 h	6 or more loose stools in 24 h	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
Nausea	No interference with activity or 1 to 2 episodes/24 h	No interference with activity or >2 episodes/24 h	Prevents daily activity, requires outpatient intravenous hydration	Emergency room visit or hospitalization for hypotensive shock
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

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
New or worsening 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

3.1.1.3 Adverse events and serious adverse events

For all dosed trial subjects in cohorts 1-8, AEs will be collected from each dose up to 1 month after this dose and for subjects in cohort 9 - from the Visit 1 (after eligibility is confirmed) up to 1 month. However, for any visit more than 1 month after each IMP injection (cohorts 1-8) or Visit 1 (cohort 9), only IMP-related AEs, trial procedure-related AEs, adverse events of special interest (AESIs), SAEs, and AEs linked to confirmed COVID-19 cases will be recorded.

Solicited AEs (i.e., reactogenicity events) that are derived from trial subjects' e-diaries should not be reported as AEs unless the event meets criteria for an SAE or starts after Day 7 or starts on Days 1 to 7 and continues past Day 7.

In addition, all solicited Grade 4 reactogenicity events should be medically confirmed.

An AE/SAE is defined as treatment-emergent adverse event (TEAE) if the event onset date and time is after the first IMP dose (if the event was absent before the first administration of the IMP) or worsened after the first IMP dose (if the event was present before the first administration of the IMP).

In the event of an incomplete onset date, the event will be considered as treatment-emergent unless the partial onset date information or complete or partial end date confirms the onset date or the event end prior to the first dose of IMP.

Missing AE date will be addressed as described in Section 5.3.

All AEs and SAEs will be coded/categorized according to terms in Medical Dictionary for Regulatory Activities (MedDRA®) version 26.0 or higher.

3.1.2 Immunogenicity endpoints – Part B

For both BNT162b2-experienced subjects and COVID-19 vaccine-naïve subjects the primary immunogenicity endpoints are:

- Reference and variant of concern (VOC) specific neutralizing titers (NTs)
- Reference and VOC specific seroresponse (SRs)

The primary immunogenicity endpoints, for subjects assigned to Part B, are evaluated against the reference at the following time points:

- VOC (B.1.1.7 and B.1.617.2) specific NTs 1 month after one dose of BNT162b2 (B.1.1.7 + B1.617.2) for subjects in Part B Cohort 1 vs reference strain NTs 1 month after two doses of BNT162b2 for subjects from the Phase III trial BNT162-02 / C4591001
- VOC (B.1.617.2) specific NTs 1 month after one dose of BNT162b2 (B1.617.2) for subjects in Part B Cohort 4 vs reference strain NTs 1 month after two doses of BNT162b2 for subjects from the Phase III trial BNT162-02 / C4591001
- Reference strain NTs 3 weeks after one dose of BNT162b2 (B.1.1.7 + B1.617.2) for subjects in Part B Cohort 6 with evidence of prior infection vs reference strain NTs 1 month after two doses of BNT162b2 for subjects without evidence of infection from the Phase III trial BNT162-02 / C4591001
- VOC (B.1.1.7 and B.1.617.2) specific NTs 1 month after two doses of BNT162b2 (B.1.1.7 + B1.617.2) for subjects in Part B Cohort 6 vs reference strain NTs 1 month after two doses of BNT162b2 for subjects from the Phase III trial BNT162-02 / C4591001. The non-inferiority test for this primary immunogenicity comparison will not be performed. The details of clinical and statistical considerations are described in Section 8.2.

Titers above the lower limit of quantitation (LLOQ) are considered accurate and their quantitated values will be presented. Values below the LLOQ will be set to 0.5× LLOQ for analysis and values above ULOQ will be set to ULOQ.

SR is defined as a ≥4-fold rise in neutralizing titer from baseline. For subjects with a baseline titer less than the lower limit of quantitation (<LLOQ), seroresponse is defined as a post-vaccination titer of ≥4 × LLOQ.

3.1.3 Immunogenicity endpoints – Part C

For subjects randomized to Part C Cohorts 7 and 8, the primary immunogenicity endpoints are:

- VOC (Omicron BA.1) specific NTs
- VOC (Omicron BA.1) specific SRs

The primary immunogenicity endpoints for subjects randomized in Part C Cohort 7 and 8 are evaluated 1 month after one dose of BNT162b2(B1.1.529) in Cohort 7 and compared to those at 1 month after one dose of BNT162b2 in Cohort 8.

3.2 Secondary endpoints

3.2.1 Immunogenicity endpoints

Part A

After one, two, or three doses of BNT162b2 (B.1.1.7 + B.1.617.2), BNT162b2 (B.1.1.7), BNT162b2 (B.1.617.2), and BNT162b2 the secondary immunogenicity endpoint is:

- Reference and VOC (B.1.1.7 and/ or B.1.617.2) specific NTs at each post-vaccination time point

Part B

For both BNT162b2-experienced subjects and COVID-19 vaccine-naïve subjects the secondary immunogenicity endpoint is:

- Reference and VOC specific NTs (B.1.1.7 and B.1.617.2) at 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) or BNT162b2 (B.1.617.2) in BNT162b2-experienced subjects vs 1 month after two doses of BNT162b2 for subjects from the Phase III trial BNT162-02 / C4591001
- Reference and VOC specific NTs (B.1.1.7 and B.1.617.2) at 1 month after two doses or three doses of BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects
- VOC specific NTs (B.1.1.7, B.1.617.2, and B.1.1.529.5) at 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects with evidence of prior infection vs 1 month after one booster dose of BNT162b2 (B.1.1.7 + B.1.617.2) in BNT162b2-experienced subjects without evidence of infection
- VOC specific NTs (B.1.1.7, B.1.617.2, B.1.1.529.5) at 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects with evidence of prior infection vs 1 month after two doses of BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects without evidence of infection

Part C

For all Cohorts in Part C of the trial, the secondary immunogenicity endpoint is:

- VOC (Omicron BA.1) specific NTs at each post-vaccination time point

3.3 Exploratory endpoints

Part A

Below are exploratory endpoints for Part A:

- Reference and VOC specific NTs (B.1.1.7 and/ or B.1.617.2) at each post-vaccination time point. This endpoint is duplicate of the secondary endpoint and the analysis will not be performed.
- CD4+ and CD8+ T-cell responses (Ex vivo ELISpot)

Part B

Below are exploratory endpoints for Part B:

- Reference and VOC (B.1.1.7 and/ or B.1.617.2) specific NTs at each post-vaccination time point
- Nucleocapsid (N)-binding antibody seroconversion in subjects with no serological or virological evidence of past SARS-CoV-2 infection or confirmed COVID-19
- Number of confirmed COVID-19 cases

Part C

Below are exploratory endpoints for Part C:

- Reference and VOC (Omicron BA.1) specific NTs at each post-vaccination time point
- Number of confirmed COVID-19 cases

The exploratory analyses not specified in this section are out of scope of this SAP and may be described in a different document.

3.3.1 Confirmed COVID-19 cases

Confirmed COVID-19 case is defined in BNT162-17 protocol v7 Section 8.2.4:

- Presence of SARS-CoV-2 NAAT-positive at a local testing facility (using an acceptable test) and COVID-19 reported AE (symptomatic).

OR

- presence of at least 1 of the symptoms as listed in the BNT162-17 protocol v7 Section 8.2.4 and
- SARS-CoV-2 NAAT-positive at the central laboratory during, 4 days before or after the symptomatic period.

The surveillance period will start post first dose of vaccination until the earliest of the following events:

- Onset date of the first confirmed COVID-19 case reported as AE.

OR

- First symptom or positive central NAAT date (whatever is earlier) identified via the above algorithm.
- When the subject's end of trial occurs due to withdrawal of consent, lost to follow-up, death, investigator/sponsor/subject decision, clinical trial completion, adverse event, protocol deviation or other.

3.3.2 Nucleocapsid-binding antibody seroconversion

Nucleocapsid (N)-binding antibody seroconversion is defined as a positive N-binding antibody result after the first dose in subjects without serological evidence of previous infection (determined by negative N-binding antibody) at Visit 1 and without previous virological evidence of infection (determined by negative NAAT results at Screening and Visit 1).

The surveillance period will start after the first dose for subjects in Cohort 1, Cohort 4, and Cohort 6 in Part B until the earliest of the following events:

- Date of the first positive N-binding antibody test.
- or when the subject's end of trial occurs.

3.3.3 CD4+ and CD8+ T-cell responses (Ex vivo ELISpot)

T cell-mediated immune responses after one, two, or three doses of the multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2), or one booster dose of the monovalent vaccine BNT162b2 (B.1.1.7), or one booster dose of BNT162b2 (B.1.617.2), at 30 µg will be analyzed. Cell-mediated immune (CMI) responses will be measured by interferon γ (IFNg) enzyme-linked immuno spot (ELISpot).

T-cell responses of the trial subjects will be analyzed for both CD4+ and CD8+ T-cells specific for the SARS-CoV-2 reference strain full length spike (FL-S), SARS-CoV-2 FLS B.1.1.7 ("Alpha variant"), and SARS-CoV-2 FLS B.1.617.2 ("Delta variant"). The SARS-CoV-2 S protein will be tested using two peptide pools (Spike protein pool 1 [SP1]: N-terminal half; Spike protein pool 2 [SP2]: C-terminal half) for each variant. Hereinafter, "target" refers to the applied experimental stimulus (spike protein pool 1, spike protein pool 2, or the sum of spike protein pools 1 + 2) in the ELISpot readout.

For analysis of responders, the spot counts of the ELISpot assay will be evaluated with the ELISpot data analysis (EDA) tool. In the next steps, these results are checked by subject matter experts, and a final call on presence or absence of T-cell responses is made for each analyzed sample, hereinafter named "expert call".

3.4 Baseline and other variables

3.4.1 Definition of baseline value and SARS-CoV-2 infection at baseline and post vaccination

Unless otherwise specified, the last non-missing measurement or sample collected prior to first IMP injection is considered the baseline data for all assessments.

Baseline in immunogenicity analysis:

The baseline value used in the derivation of geometric mean fold rise (GMFR), SR, and difference of SR for Part A, Part B and Part C of the trial is the value before first dose of trial IMP.

Baseline SARS-CoV-2 infection status:

For Part A and Part B, baseline SARS-CoV-2 infection is defined as below:

Positive:

- if any one of the 3 tests, i.e., nucleocapsid-binding antibody seroconversion, central NAAT, or local NAAT, is positive,

  OR

- had medical history (MH) or AE of COVID-19 prior to vaccination.

Negative:

- no MH or AE of COVID-19 prior to vaccination,

  AND

- negative N-binding.

AND

- both local and central NAAT negative or one negative and one missing.

Missing/indeterminate:

- no MH or AE of COVID-19 prior to vaccination,

  AND

- no positive test results from any of the 3 tests,

  AND

- N-binding missing or both NAAT missing.

Evidence of SARS-CoV-2 infection post vaccination

No evidence of SARS-CoV-2 infection post vaccination for subjects in Part B is defined as subjects who had no serological or virological evidence of SARS-CoV-2 infection (i.e., negative N-binding antibody [serum] result and negative NAAT [oral swab] at all planned visits and any unscheduled visit (up to the 1-month post–Dose 2 blood sample collection for Cohort 6 and 1-month post–trial vaccination blood sample collection for Cohort 1 and Cohort 4) and had no medical history or AE of COVID-19 (up to the 1-month post–Dose 2 blood sample collection for Cohort 6 and 1-month post–trial vaccination blood sample collection for Cohort 1 and Cohort 4)).

No evidence of SARS-CoV-2 infection post vaccination for subjects from Phase III trial BNT162-02 / C4591001 is defined as subjects who had no serological or virological evidence (up to the 1-month post–Dose 2 blood sample collection) of SARS-CoV-2 infection (i.e., negative N-binding antibody [serum] result at the Dose 1 and 1-month post–Dose 2 visits, negative NAAT [nasal swab] at the Dose 1 and Dose 2 visits, and any unscheduled visit (up to the 1-month post–Dose 2 blood sample collection)) and had no medical history of COVID-19.

3.4.2 Trial day and analysis visits

Trial day:

Trial day will be calculated in reference to Day 1, defined as the date of first dose of trial IMP administration. For assessments conducted after Day 1, trial day is calculated as (assessment date – Day 1 date +1). For assessments conducted before Day 1, trial day is calculated as (assessment date – Day 1 date). There will be no Trial Day 0.

Analysis visits:

For clinical safety data summary, lab data, vital signs, electrocardiogram (ECG), and physical exam, the analysis visits post vaccination will be based on the visits reported and validated in the EDC database.

For immunogenicity assays, the sampling windows with grace period specified in Table 7 will be used to define the valid samples to include in the Immunogenicity Analysis Set, and the per protocol sampling windows will be used to define the valid samples to include in the Immunogenicity Analysis Per Protocol Set. See Table 8 for the definitions of the analysis sets.

Table 7. Immunogenicity sampling windows

Visit relative to dose	Window per CTP = Include in (per protocol) Immunogenicity Analysis Set	Additional grace period = No impact on immunogenicity = Include in Immunogenicity Analysis Set	Impact on immunogenicity = Exclude from Immunogenicity Analysis Set Important PD if supporting a primary endpoint. Non-important PD if not supporting a primary endpoint
1 week post dose	8+2 8-10 days	NAP	All OOW
3 weeks post dose	21±3 18-24 days	+/- 2 x days OOW ≥16 to ≤26 days	+/- 3 days and more OOW <16 days or >26 days
1 month post dose	29±3 26-32 days	+/- 7 days OOW ≥19 to ≤39 days	+/- 8 and more days OOW <19 days or >39 days
3 months post dose	85±5 80-90 days	+/- 7 x days OOW ≥73 to ≤97 days	+/- 8 and more days OOW <73 days or >97 days
6 months post dose	180±10 170-190 days	+/- 7 x days OOW ≥163 to ≤197 days	+/- 8 and more days OOW <163 days or >197 days
1 year post dose	360±10 350-370 days	+/- 14 x days OOW ≥336 to ≤384 days	+/- 15 and more days OOW <336 days or >384 days

Grace period will not be applied.
Abbreviations: OOW = out of window; NAP = not applicable; PD = protocol deviation.

The following conversion rules will be used to convert days to months and days to years:

- 1 month = 30.4375 days
- 1 year = 365.25 days

For subjects in Part A and Part B (Cohort 1 and Cohort 4) of the trial, time at IMP Dose 1 (Vax 3) since Dose 2 of BNT162b2 (Vax 2) in days = (Date of IMP Dose 1 - Date of BNT162b2 vaccine Dose 2) +1. BNT162b2-experienced subjects have received BNT162b2 vaccine (30 µg, two-dose regimen) in either a clinical trial (e.g., BNT162-02/C4591001) or as part of the governmental vaccination programs.

3.4.3 Demographics, medical history, and physical examination

The demographic variables are age at screening (in years), age group for Part B and Part C (18 to 55 years, 56 to 85 years, 18 to 64 years, and 65 to 85 years), sex (Male or Female), childbearing potential (yes or no), ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not Reported and Unknown), race (Black or African American, American Indian or Alaskan Native, Asian, Native Hawaiian or other Pacific Islander, White, Other, Multiracial,

Not reported and Unknown), country, height(cm), body weight(kg), Body Mass Index (BMI (kg/m^2), Underweight (below 18.5), Normal weight (18.5 to 24.9), Over weight (25 to 29.9), Obese (30 and above)), and diabetes mellitus (Yes or No), baseline SARS-CoV-2 infection status (Positive or Negative) and Time from the second dose of BNT162b2 (received prior to the trial) to the first trial vaccination (days).

In cases where more than 1 category is selected for race, the subject would be counted under the category "multiracial" for analysis.

For Part C, when the last SARS-CoV-2 infection prior to the IMP dose was diagnosed (time between infection and trial randomization), the number of prior COVID-19 RNA vaccines and time from the last dose of COVID-19 RNA vaccination (received prior to the trial) to trial randomization will be presented.

Medical history (MH) will be categorized according to MedDRA® version 24.0 or higher.

Physical examinations will be performed evaluating any clinically significant abnormalities. Body weight (in kg) and any clinically significant abnormal results will be recorded in the case report form (CRF).

Vital signs (oral body temperature, heart rate, blood pressure [systolic and diastolic]), blood and urine clinical laboratory tests will also be recorded.

3.4.4 Prior/Concomitant medications and concomitant procedures

Prior/concomitant medications and concomitant procedures (including non-drug therapies) will be recorded in the CRF. Medications and procedures will be assigned as being prior to trial IMP, concomitant with IMP based on the start and stop dates of the medication and dosing dates.

If the medication/procedure taken from screening visit and stopped before the date of the first dose of trial IMP, the medication/procedure will be assigned as being prior to trial IMP. Otherwise, if taken after the first dose of trial IMP, the medication/procedure will be assigned as being concomitant with trial IMP.

All medications will be coded using the latest version of World Health Organization (WHO) Drug Dictionary.

3.4.5 Other safety endpoints

12-lead ECG will be recorded before and at 7 days after each IMP injection and any abnormal results will be recorded in the CRF. Clinically significant ECG abnormalities will be captured as AEs.

4 ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all subjects will be assessed to determine if they meet the criteria for inclusion in each analysis set. All analysis sets will be assessed and documented prior to releasing the database for each cohort.

For all trial parts, analyses of safety endpoints will be performed using the Safety Set. Subjects will be summarized for safety analysis according to the vaccine group they actually received. For Part C, demographic and baseline characteristics, medical history,

and prior medications will be tabulated for all randomized subjects as well. Analyses of primary, secondary and exploratory immunogenicity endpoints will be performed using Immunogenicity Analysis Set, Immunogenicity Analysis Per Protocol Set, and/or All-available Immunogenicity Set (mITT) as specified in Sections 6.1.2, 6.2, and 6.3. For mITT, subjects will be summarized according to the vaccine group to which they were randomized/assigned. The analyses of reactogenicity endpoints (i.e., local reactions and systemic events) will be based on Reactogenicity Set. The definitions of each analysis set are specified below in Table 8.

Table 8. Analysis set

Analysis set	Description
Screened Set	All subjects who signed informed consent.
All Randomized Subjects	All subjects who were randomized to Cohort 7, 8, 9 in Part C
Safety Set	All subjects who received at least one dose of IMP.
Reactogenicity Set	All subjects included in the Safety Set with any e-diary data reported after IMP injection.
Immunogenicity Analysis Set	All eligible randomized/assigned subjects who receive the trial intervention to which they are randomized or assigned, have a valid and determinate immunogenicity result from the blood sample collected within an appropriate window[a], and have no other important protocol deviations that can confound immunogenicity data [c,d].
Immunogenicity Analysis Per Protocol Set	All eligible randomized/assigned subjects who receive the trial intervention to which they are randomized or assigned, have a valid and determinate immunogenicity result from the blood sample collected within per protocol window[b], and have no other important protocol deviations that can confound immunogenicity data[c,d].
All-available Immunogenicity Set (mITT)	All randomized/assigned subjects who receive the trial intervention and have a valid and determinate immunogenicity result after the trial intervention.

An important protocol deviation is a protocol deviation that, in the opinion of the sponsor's clinician, would materially affect assessment of immunogenicity, e.g., subject receipt of a prohibited vaccine or medication that might affect immune response or a medication error with suspected decrease in potency of the vaccine.

a) The sampling windows with grace period specified in Table 7 will be used to define the valid samples for Immunogenicity Analysis Set.
b) The sampling windows per protocol specified in Table 7 will be used to define the valid samples for Immunogenicity Analysis Per Protocol Set.
c) In cohort 9 all randomized subjects who have a valid and determinate immunogenicity result from the blood sample and have no other important protocol deviations that can confound immunogenicity data, will be included in the Immunogenicity Analysis Set.
d) Participants with missing baseline data will be included in this population, providing other criteria are met

Abbreviation: IMP = investigational medicinal product.

5 GENERAL METHODOLOGY AND CONVENTIONS

Unless otherwise specified, all statistical analysis will be performed by Cohort and separately by trial parts. For subjects in Part B and Part C of the trial, summaries will also be presented by age-group 18-55 years and 56-85 years. The primary immunogenicity analysis for each Part B and Part C Cohort will be performed after completion of 1 month or 3 weeks post-dose visit. All other data may be analyzed periodically.

For data analysis of local reactions and systemic events, reactogenicity events (i.e., local reactions and systemic events) within 7 days of trial IMP injection will be included in the tables, figures and listings.

5.1 Hypotheses and decision rules

Statistical hypotheses will only be tested in Part B, separately for each cohort.

Immunobridging success in each Part B Cohort requires demonstration of non-inferior antibody levels in both geometric mean titers (GMTs) and SR rates against the B.1.1.7 and B.1.617.2 variants of concern (VOC) in both Cohorts 1 and 6; and against B.1.617.2 in Cohort 4 after the booster dose vs antibody levels against the reference strain, observed in subjects from the Phase III BNT162-02/C4591001 trial, after two doses of BNT162b2. In addition, non-inferiority of immune response against reference strain 21 days after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) in Cohort 6 subjects with prior evidence of infection vs immune response against reference strain 1 month after two doses of BNT162b2 in subjects without evidence of infection will also be assessed using the same success criteria.

5.1.1 GMT non-inferiority – Part B

The statistical hypothesis for GMT non-inferiority of each variant and reference strain is:

$$H0: \mu_{ratio} \leq 0.67 \text{ vs } H1: \mu_{ratio} > 0.67$$

where 0.67 corresponds to a 1.5-fold margin for non-inferiority, and μ_{ratio} is the ratio of GMT of VOC specific NT at 1 month after booster dose of BNT162b2 (B.1.1.7 + B.1.617.2) for Cohort 1 and BNT162b2 (B.1.617.2) for Cohort 4 to the reference NT at 1 month after second dose of BNT162b2 or ratio of GMT of reference strain NT at 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) to 1 month after the second dose of BNT162b2 (defined as the geometric mean ratio [GMR]).

GMT VOC non-inferiority success for Cohort 1 will be declared if the lower bound of the 2-sided 95% confidence intervals (CIs) for (1) $GMR_{B.1.1.7/ref}$ (GMT of B.1.1.7 ÷ GMT of

reference) and (2) GMR $_{B.1.617.2/ref}$ (GMT of B.1.617.2 ÷ GMT of reference) are both greater than 0.67. GMT reference strain non-inferiority success for Cohort 6 will be declared if the lower bound of the 2-sided 95% CI for $GMR_{ref/ref}$ (GMT of reference ÷ GMT of reference) is greater than 0.67. GMT VOC non-inferiority success for Cohort 4 will be declared if the lower bound of the 2-sided 95% confidence interval GMR $_{B.1.617.2/ref}$ (GMT of B.1.617.2 ÷ GMT of reference) is greater than 0.67.

5.1.2 SR non-inferiority – Part B

The statistical hypothesis for SR non-inferiority for each variant and reference strain is:

$$H0: \pi_{variant} - \pi_{reference} \leq -10\% \text{ vs}$$

$$H1: \pi_{variant} - \pi_{reference} > -10\%$$

where $\pi_{variant}$ is the SR against the VOC at 1 month after booster dose of BNT162b2 (B.1.1.7 + B.1.617.2) for Cohort 1 and BNT162b2 (B.1.617.2) for Cohort 4 or SR against the reference strain at 3 weeks after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) and $\pi_{reference}$ is the SR against the reference strain at 1 month after second dose of BNT162b2.

The SR VOC non-inferiority success for Cohort 1 will be declared if the lower bounds of the 2-sided 95% CIs for the difference of:

1. SR of B.1.1.7 – SR of reference and
2. SR of B.1.617.2 – SR of reference

are both greater than -10%. The SR reference strain non-inferiority success for Cohort 6 will be declared if the lower bounds of the 2-sided 95% CIs for the difference in SRs of reference is greater than -10%. The SR VOC non-inferiority success for Cohort 4 will be declared if the lower bound of the 2-sided 95% CI for the difference of SR of B.1.617.2 – SR of reference is greater than -10%.

5.1.3 Sample size

Planned enrollment is ~1,470 subjects; ~120 in Part A (~20 in each of the six Cohorts), ~1,125 in Part B (~375 in each of the three Cohorts), and ~225 in Part C (~80 in each of Cohort 7 and Cohort 8 and ~45 in Cohort 9).

5.1.3.1 Part A

The sample size for Part A of the trial is not based on any statistical hypothesis testing. Part A comprises 20 subjects per Cohort, corresponding to a total of 120 subjects.

5.1.3.2 Part B

Geometric mean ratio

Assuming an observed GMR of 0.8 and standard deviation of 0.7 (log scale), 300 evaluable subjects will provide 87% power at alpha = 0.025 (1-sided) to demonstrate non-inferiority of VOC vs reference strain response. The table below demonstrates the power for testing GMT for each variant strain vs reference strain at alpha = 0.025 (1-sided):

Test	Margin	Assumed GMR	Number of evaluable subjects	SD log scale	Power
VOC vs reference strain GMT non-inferiority	0.67	0.8	300	0.65	92%
				0.70	87%
				0.75	82%
Reference strain vs reference strain GMT non-inferiority	0.67	0.8	240	1.05	46%
		0.9	240	1.05	87%
		1.0	240	1.05	99%

Abbreviations: GMT = geometric mean titer; GMR = geometric mean ratio; SD = standard deviation; VOC = variant of concern.

Seroresponse

Assuming an SR difference of -3% (variant SR – reference SR) and 97% reference SR, 300 evaluable subjects will provide 96% power at alpha = 0.025 (1-sided). The table below demonstrates the power for testing SR for each variant strain vs reference strain at alpha = 0.025 (1-sided) to demonstrate non-inferiority of VOC vs reference strain response.

Test	Margin	SR difference	Number of evaluable subjects	Reference SR	Power
VOC vs reference strain SR non-inferiority	-10%	-3%	300	98%	98%
				97%	96%
				95%	90%
Reference strain vs reference strain SR non-inferiority	-10%	-3%	240	98%	95%
				97%	91%
				95%	83%
	-10%	-5%	240	98%	65%
				97%	59%
				95%	50%

Margin is the lower bound of 95% CI.

Abbreviations: CI = confidence interval; SR = seroresponse; VOC = variant of concern.

Therefore, to account for a 20% non-evaluable rate, approximately 375 subjects will be enrolled for each cohort.

In Cohort 6, assuming 80% subjects with evidence of prior infection, 240 evaluable subjects will contribute to the hypotheses for non-inferiority of immune response against reference strain. Tables above also present the power to demonstrate non-inferiority of immune response against reference strain under various assumptions in terms of GMT and SR.

5.1.3.3 Part C

No confirmatory hypothesis testing is planned for Part C. However, exploratory analysis may be performed on selected endpoints. To describe the immune profile, 80 evaluable subjects per Cohort in Cohorts 7 and 8 and 40 evaluable subjects in Cohort 9 are

considered adequate based on experience in previous trials conducted by BioNTech in the BNT162 program.

Geometric mean ratio

The table below demonstrates the power for testing GMT for variant strain post BNT162b2 vs post BNT162b2 (B.1.1.529) at alpha = 0.025 (1-sided) to demonstrate superiority.

Test	Margin	Assumed GMR (GMT variant ÷ GMT ref)	Number of evaluable subjects	Standard deviation (log value)	Power
GMT superiority	1.0	1.75	80	1.0	94%

Margin is the lower bound of 95% CI for GMR.
Abbreviations: GMT = geometric mean titer; GMR = geometric mean ratio.

To account for ~11% non-evaluable rate, Part C will randomize 225 subjects in a 2:2:1 ratio (90 each for Cohorts 7 and 8 and 45 in Cohort 9).

5.1.4 Multiplicity considerations

5.1.4.1 Part A

For Part A, there is no hypothesis testing.

5.1.4.2 Part B

The objectives for Cohort 1, 4, and 6 will be evaluated independently. Subjects in each cohort are different populations. Therefore, no type I error adjustments will be applied to the assessments across the three cohorts. A fixed sequential testing procedure will be used for multiplicity control for each cohort separately across Part B, all at 1-sided 2.5% level of significance.

For Cohort 1 and Cohort 4, the hypotheses will be tested in the following sequence:

1. Non-inferiority in terms of GMTs
2. Non-inferiority in terms of SRs

For Cohort 6, the hypotheses will be tested in the following sequence:

1. Non-inferiority of immune response against reference strain in terms of GMT
2. Non-inferiority of immune response against reference strain in terms of SR
3. Non-inferiority of immune response against VOCs vs reference strain in terms of GMTs
4. Non-inferiority of immune response against VOCs vs reference strain in terms of SRs

5.1.4.3 Part C

No confirmatory hypothesis testing is planned for Part C.

5.2 General methods

All statistical procedures will be completed using SAS version 9.4 or higher. Unless otherwise specified, 2-sided 95% CIs for all endpoints in the statistical analysis, will be presented. Data assessed after the vaccination with a non-trial COVID-19 vaccine will be excluded from all immunogenicity analyses.

Analysis of antibody titers will be performed on natural log scale and the results will be exponentiated back to original scale.

For each subject and each time point two titers for each variant will be determined, as each sample will be measured in replicate. The response per subject, variant, and time point is defined as the geometric mean of the two titers.

All relevant subject data, including those derived will be presented in individual subject data listings.

5.2.1 Analyses for binary data

Descriptive statistics for categorical variables (e.g., proportions) are the percentage (%), with the number of subjects with data on the specified characteristic as the numerator (n), and the number of subjects in the specific analysis set as denominator (N) used in the percentage calculation, and the 95% CIs where applicable.

The exact 95% CI for binary endpoints for each group will be computed using the F distribution (Clopper-Pearson; Agresti 2002). Unless otherwise stated, the 95% CI for the between-group difference for binary endpoints will be calculated using the Miettinen and Nurminen method (Miettinen and Nurminen 1985).

For between-group comparison of SR, the difference will be estimated using minimum risk weights and stratified by sex and age group (18 to 55 years, 56 to 85 years). The associated 95% CI will be calculated using stratified Newcombe confidence interval weighted with minimum risk to combine the stratum components and continuity correction.

5.2.2 Analyses for count data

The number of occurrences of a certain event is count data and thus could be modeled using Poisson distribution. The incidence rate is estimated as the number of events observed divided by the total person-years of follow-up.

Assuming an observed event is from Poisson distribution with parameter λT, where λ is the incidence rate and T is the total person-years of follow-up, based on the relationship between the Poisson and chi-square distribution, the exact lower (l) and upper (u) α-percent 2-sided confidence limits for λT can be estimated by:

$Y_l = \frac{\chi^2_{2Y,\alpha/2}}{2}$ and $Y_u = \frac{\chi^2_{2(Y+1),1-\alpha/2}}{2}$, respectively,

where Y is the number of events observed.

The exact lower and upper confidence limit for incidence rate λ can then be obtained as Y_l/T and Y_u/T, respectively.

5.2.3 *Analyses for continuous data*

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

5.2.3.1 Geometric means

For immunogenicity results the GMTs will be computed along with associated 95% CIs. The geometric mean on NT for each cohort will be calculated separately for all trial parts by exponentiating the mean logarithm of the titers and corresponding CIs based on the Student's t-distribution. Assay results below the LLOQ are set to 0.5 × LLOQ.

5.2.3.2 Geometric mean fold rises

Geometric mean fold rise (GMFR) will be defined as the result after vaccination divided by the baseline result. GMFRs are limited to subjects with non-missing values at both time points.

GMFRs will be calculated as the mean of the difference of logarithmically transformed neutralization titers or antibody levels (later result minus earlier result) and exponentiating the mean. The associated 2-sided 95% CIs are obtained by constructing CIs using Student's t-distribution for the mean difference on the natural log scale and exponentiating the confidence limits.

5.2.3.3 Geometric mean ratios

For the between-group comparison, adjusted GMRs and the associated 95% CIs will be calculated by exponentiating the difference of least square means and corresponding CIs based on the analysis of logarithmically transformed neutralizing titers. A linear regression model with age, sex, and group (each Part B Cohort versus reference group) will be done. Unadjusted GMRs and the associated 2 sided 95% CIs will be calculated by exponentiating the mean difference of the logarithms of the titers and the corresponding CI (based on the Student's t-distribution).

5.3 Methods to manage missing data

Unless otherwise specified, data in summary tables will be presented using Observed Case (OC) data and therefore no missing data will be imputed.

Missing binary and continuous data will be presented only if there is a non-zero count for at least one of the Cohorts.

For endpoints, the missing data handling rules are described in the corresponding endpoint sections.

Handling missing data:

Missing data, other than that described for AE and CM below will not be imputed.

For the purposes of assigning treatment-emergent flag for AEs, partial or missing AE dates will be handled as follows:

- If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as trial IMP. In this case, to conservatively report the event as treatment-emergent, the onset date will be assumed to be the first date of trial IMP.
- If the onset day and month are both missing, the day and month will be assumed to be 01 January, unless the event occurred in the same year as the trial IMP. In this case, the event onset will be assumed to be the day and month of IMP to conservatively report the event as treatment emergent.
- A completely missing onset date will be assumed to be the first day of trial IMP.

For the purposes of assigning prior or concomitant flag for medications (CM), partial or missing medication dates will be handled as follows:

- If end day is missing and month/year are non-missing, then day is the minimum of treatment end date and the last day of the month.
- If end day/month are missing and year is non-missing, then day is the minimum of treatment end date and the end of the year (31DECYYYY).
- If imputed end date is less than the start date, use the start date as the imputed end date.

Start dates:

- If the start date year is missing, the start date is set to one day prior to treatment start date.
- If the start date year is less than the treatment start date year, then:
  - If the month is missing, the start date is assumed to be mid-year point (01JULYYYY).
  - Else if the month is not missing, the start date is assumed to be mid-month point (15MONYYYY).
- If the start date year value is greater than the treatment start date year, then:
  - If the month is missing, the start date is assumed to be the year start point (01JANYYYY).
  - Else if the month is not missing, the start date is assumed to be the month start point (01MONYYYY).
- If the start date year value is equal to the treatment start date year value:
  - If the month is missing or the month is equal to the treatment start date month, then the start date is assumed to be one day prior to the treatment start date.
  - Else if the month is less than the treatment start date month, the start date is assumed to the mid-month point (15MONYYYY).
  - Else if the month is greater than the treatment start date month, the start date is assumed to be the month start point (01MONYYYY).

For both AE and CM, if complete end date is available and the start date assumed from the steps above is greater than the end date, then the assumed start date should be set to the end date.

6 ANALYSES AND SUMMARIES

6.1 Primary endpoints

6.1.1 Safety endpoints (Part A, Part B and Part C)

If not specified otherwise, all safety analyses will be based on the Safety Set and summarized by Cohort and trial part. The summary analyses post dose 2 and post dose 3 of IMP will be performed with subjects who received two doses and three doses of IMP to calculate the percentages.

6.1.1.1 Safety overview

The number of events, number and percentage of subjects reporting at least one of the below events from IMP administration will be summarized by treatment group post each dose and combined total of events for the periods from each dose up to 1 month after each dose based on Safety Set.

- Solicited local reactions and systemic events
- Any TEAE
- Related TEAE
- TEAE with Grade ≥3
- Related TEAE with Grade ≥3
- Any solicited AE that starts on Days 1 to 7 and continues longer than 7 d post-vaccination or starts after Day 7 or is an SAE
- Any SAE
- Related SAE
- SAE with Grade ≥3
- Related SAE with Grade ≥3
- TEAE leading to trial withdrawal
- TEAE leading to treatment withdrawal
- Any AESI
- All cause death

6.1.1.2 Local reactions

The number and percentage of subjects reporting local reactions (pain at the injection site, tenderness, erythema/redness, induration/swelling) within 7 days after each dose will be

summarized by maximum severity and cumulatively across severity levels. The associated 2-sided Clopper-Pearson 95% CI's will also be displayed.

The Reactogenicity Set will be used for local reaction summaries. Subjects without any e-diary data throughout the 7 days after vaccination or have recorded events only beyond 7 days, will be excluded from the local reaction analysis.

In addition, local reactions will also be summarized by:

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

Bar charts with the proportions of subjects for each local reaction throughout 7 days after each dose will be plotted. The bars will be divided into severity categories to highlight the proportions of subjects by maximum severity. In addition, listing of subjects in the Reactogenicity Set, with reported local reactions, will be provided.

6.1.1.3 Systemic events

Systemic events (fever, fatigue, headache, chills, vomiting, nausea, diarrhea, new or worsened muscle pain, and new or worsening joint pain) reported within 7 days after each dose will be summarized in the same manner as local reactions (see Section 6.1.1.1) using the Reactogenicity Set. Subjects without any e-diary data throughout the 7 days after vaccination will be excluded from the systemic events analysis. In addition, listing of subjects in the Reactogenicity Set, with reported systemic events, will be provided.

6.1.1.4 Adverse events

The number of events, number and percentage of subjects reporting a treatment-emergent adverse event (TEAE) up to 1 month post each dose will be summarized by system organ class (SOC) and preferred term (PT) in descending order of frequency within both SOC and PT. Where a subject has the same TEAE, based on SOC or PT, reported multiple times in the same category the subject will only be counted once at that system organ class/preferred term.

6.1.1.5 Serious adverse events

The number of events, number and percentage of subjects reporting serious TEAEs and serious related TEAEs up to 6 months after last dose will be summarized in the same manner as the AEs. The non-serious AEs will also be summarized.

6.1.2 Immunogenicity endpoints – Part B

Between-group comparison of Part B Cohort 1, 4 and 6 with subjects from the Phase III trial BNT162-02 / C4591001 trial

To evaluate the primary immunogenicity endpoints for Part B Cohort 1, 4 and 6 using non-inferiority test, separate control groups, one for each cohort in Part B, will be selected from the BNT162-02 / C4591001 trial who received two doses of BNT162b2 and had corresponding immune results at 1 month post 2nd dose. Subjects will only be selected into one of the control groups. Control groups will be randomly selected with matching factors sex and age (in years) to ensure comparable distribution in each cohort. The subject selection is described in Appendix 2.

6.1.2.1 GMR of VOC specific NT at 1 month after 1 dose BNT162b2 (B.1.1.7 + B.1.617.2) in BNT162b2 experienced subjects vs reference strain NT at 1 month after 2 doses of BNT162b2 (Cohort 1 vs subjects from the Phase III trial BNT162-02 / C4591001)

The number of subjects with valid and determinate assay results for the specified assay at each given dose/sampling time point, GMT, GMR and the associated 2-sided 95% CIs will be provided for each of Cohort 1 VOC (B.1.1.7 + B.1.617.2) strains vs subjects from the Phase III trial BNT162-02/C4591001 reference strain.

GMRs and associated 2-sided 95% CIs will be calculated using linear regression model as described in Section 5.2.3.3.

Below sensitivity analyses may be performed as appropriate:

1. Unadjusted GMR and 95% CI based on student t-distribution using the Immunogenicity Analysis Set.
2. Adjusted GMR and 95% CI in subjects, without evidence of SARS-CoV-2 infection up to one-month after 1 dose of BNT162b2 (B.1.1.7 + B.1.617.2) and up to one-month after the second dose of BNT162b2, with age (in years) and sex as covariates using the Immunogenicity Analysis Set. No evidence of SARS-CoV-2 infection post vaccination is defined in Section 3.4.1.
3. Also, bar plots including LLOQ and ULOQ lines and with different color codes for data from subjects according to their N-binding assessment for the respective visit, time point will be presented.

6.1.2.2 The difference in SRs to VOC specific NT 1 month after 1 dose BNT162b2 (B.1.1.7 + B.1.617.2) to the reference strain NT at 1 month after 2 doses in BNT162b2 (Cohort 1 vs subjects from the Phase III trial BNT162-02 / C4591001)

The number and percentage of subjects with SR at each specified time point and the difference in percentages with 95% CI will be provided for each of Cohort 1 VOC (B.1.1.7 + B.1.617.2) strains and subjects from the Phase III trial BNT162-02/C4591001 reference strain.

Adjusted difference in proportions will be estimated using minimum risk weights and stratified by sex and age group (18 to 55 years, 56 to 85 years) and expressed as a percentage. The associated 2-Sided CI will be based on the Newcombe method stratified by sex and age group (18 to 55 years, 56 to 85 years) with minimum risk weights for the difference in proportions.

Non-inferiority will be assessed sequentially for GMR followed by SR. Non-inferiority will be declared if the lower bound of the 2-sided 95% CI for the difference in percentages of participants with SR is greater than -10%.

Below sensitivity analysis may be performed as appropriate:

1. Unadjusted differences of SR using the Immunogenicity Analysis Set will be calculated as the difference in SRs between VOC and reference. The 95% CI will be obtained using Miettinen and Nurminen Confidence Limits.

2. Adjusted difference in SR for subjects, without evidence of SARS-CoV-2 infection up to one-month after 1 dose of BNT162b2 (B.1.1.7 + B.1.617.2) and up to one month after the second dose of BNT162b2 with age group (18 to 55 years, 56 to 85 years) and sex as covariates using the Immunogenicity Analysis Set.

6.1.2.3 GMR of VOC specific NT at 1 month after 1 dose BNT162b2 (B.1.617.2) vs reference strain NT at 1 month after 2 doses of BNT162b2 (Cohort 4 vs subjects from the Phase III trial BNT162-02 / C4591001)

The number of subjects with valid and determinate assay results for the specified assay at each given dose/sampling time point, GMT, GMR and the associated 2-sided 95% CIs will be provided for Cohort 4 VOC (B.1.617.2) strain vs subjects from the Phase III trial BNT162-02/C4591001 reference strain.

GMRs and associated 2-sided 95% CIs will be calculated using linear regression model as described in Section 5.2.3.3.

Below sensitivity analyses may be performed as appropriate:

1. Unadjusted GMR and 95% CI based on student t-distribution using the Immunogenicity Analysis Set.
2. Adjusted GMR and 95% CI subjects, without evidence of SARS-CoV-2 infection up to one-month after 1 dose of BNT162b2 (B.1.617.2) and up to one month after the second dose of BNT162b2 with age (in years) and sex as covariates using the Immunogenicity Analysis Set.

Also, bar plots including LLOQ and ULOQ lines and with different color codes for data from subjects according to their N-binding assessment for the respective visit, time point will be presented.

6.1.2.4 The difference in SRs to VOC specific NT 1 month after 1 dose BNT162b2 (B.1.617.2) to the reference strain NT at 1 month after 2 doses in BNT162b2 (Cohorts 4 vs subjects from the Phase III trial BNT162-02 / C4591001)

The number and percentage of subjects with SR at each specified time point and the difference in percentages with 95% CI will be provided for Cohort 4 VOC (B.1.617.2) strain and subjects from the Phase III trial BNT162-02/C4591001 reference strain.

The adjusted difference in percentages will be estimated using minimum risk weights and stratified by sex, age group (18 to 55 years, 56 to 85 years). The associated 95% CI will be calculated using stratified Newcombe confidence interval weighted with minimum risk to combine the stratum components and continuity correction.

Non-inferiority will be assessed sequentially for GMR followed by SR. Non-inferiority will be declared if the lower bound of the 2-sided 95% CI for the difference in percentages of participants with SR is greater than -10%.

Below sensitivity analysis may be performed as appropriate:

1. Unadjusted differences of SR using the Immunogenicity Analysis Set will be calculated as the difference in SRs between VOC and reference. The 95% CI will be obtained using Miettinen and Nurminen Confidence Limits.

2. Adjusted difference in SR for subjects, without evidence of infection up to one-month after 1 dose of BNT162b2 (B.1.617.2) and up to one month after the second dose of BNT162b2 with age group (18 to 55 years, 56 to 85 years) and sex as covariates using the Immunogenicity Analysis Set.

6.1.2.5 GMR of reference strain NT at 3 weeks after 1 dose of BNT162b2 (B.1.1.7 + B1.617.2) vs reference strain NT at 1 month after 2 doses of BNT162b2 in COVID-19 vaccine-naïve subjects (Cohort 6 with prior infection vs BNT162-02 / C4591001 without infection)

The number of subjects with valid and determinate assay results for the specified assay at each given dose/sampling time point, GMT, GMR and the associated 2-sided 95% CIs will be provided.

Adjusted GMRs and associated 2-sided 95% CIs will be calculated using linear regression model as described in Section 5.2.3.3. Non-inferiority will be declared if the lower bound of the 2-sided 95% CI for GMR is >0.67.

Two sensitivity analyses will be performed:

1. Unadjusted GMR and 95% CI based on the student t-distribution using the Immunogenicity Analysis Set.
2. Unadjusted GMR and 95% CI based on the student t-distribution using the All-available Immunogenicity Set (mITT), if there is a meaningful difference in the populations.

6.1.2.6 SR to reference strain NT 3 weeks after 1 dose of BNT162b2 (B.1.1.7 + B1.617.2) vs SR to the reference strain NT at 1 month after 2 doses of BNT162b2 in COVID-19 vaccine-naïve subjects (Cohort 6 with prior infection vs BNT162-02 / C4591001 without infection)

The number and percentage of subjects with SR at each specified time point and the difference in percentages with 95% CI will be provided.

The adjusted difference in percentages will be estimated using minimum risk weights and stratified by sex and age group (18 to 55 years, 56 to 85 years). The associated 95% CI will be calculated using stratified Newcombe confidence interval weighted with minimum risk to combine the stratum components and continuity correction.

Non-inferiority will be assessed sequentially for GMR followed by SR. Non-inferiority will be declared if the lower bound of the 2-sided 95% CI for the difference in percentages of participants with SR is greater than -10%.

Two sensitivity analyses will be performed:

1. Unadjusted difference in SR and the 95% CI calculated using Miettinen and Nurminen method on Immunogenicity Analysis Set.
2. Unadjusted difference in SR and the 95% CI calculated using Miettinen and Nurminen method on All-available Immunogenicity Set (mITT), if there is a meaningful difference in the populations.

6.1.2.7 GMR of VOC specific NT at 1 month after 2 doses vs reference strain NT at 1 month after 2 doses in COVID-19 vaccine-naïve subjects (Cohort 6 vs subjects from the Phase III trial BNT162-02 / C4591001)

The non-inferiority test for this primary immunogenicity endpoint and associated sensitivity analyses will not be performed. The details of clinical and statistical considerations are described in Section 8.2.

6.1.2.8 SR to VOC specific NT 1 month after 2 doses vs SR to the reference strain NT at 1 month after 2 doses in COVID-19 vaccine-naïve subjects (Cohort 6 vs subjects from the Phase III trial BNT162-02 / C4591001)

The non-inferiority test for this primary immunogenicity endpoint and associated sensitivity analyses will not be performed. The details of clinical and statistical considerations are described in Section 8.2.

6.1.3 Immunogenicity endpoints – Part C

6.1.3.1 GMR of VOC specific NT at 1 month after 1 dose of BNT162b2 (B.1.1.529) to those at 1 month after one dose of BNT162b2 for Cohorts 7 and 8

The number of subjects with valid and determinate assay results for the specified assay at each given dose/sampling time point, GMT, GMR and 95% CI by Cohort the associated 2-sided 95% CIs will be provided.

The between group comparison using linear regression model with age and number of prior doses as covariates based on Immunogenicity Analysis Set will be done.

1. Unadjusted GMR will be calculated without age and number of doses based on the student t-distribution using Immunogenicity Analysis Set as sensitivity analysis.

6.1.3.2 SR to VOC specific NT at 1 month after 1 dose of BNT162b2 (B.1.1.529) vs SR to those at 1 month after one dose of BNT162b2 for Cohorts 7 and 8

The number and percentage of subjects with SR at each time point and the difference in percentages with 95% CI will be provided by Cohort.

The adjusted difference in percentages will be estimated using minimum risk weights and stratified by age group and number of prior doses based on Immunogenicity Analysis Set. The associated 95% CI will be calculated using stratified Newcombe confidence interval weighted with minimum risk to combine the stratum components and continuity correction.

1. Unadjusted SR will be calculated without age and number of doses and the 95% CI will be calculated using Miettinen and Nurminen method on Immunogenicity Analysis Set as sensitivity analysis.

6.2 Secondary endpoints

6.2.1 Immunogenicity endpoints – Part A

6.2.1.1 GMT and GMFR of Reference and VOC specific NT at each specified post-vaccination time point

The number of subjects with valid and determinate assay results for the specified assay at each given dose/sampling time point, GMT, GMFRs, and the associated 2-sided 95% CIs will be provided by Cohort based on Immunogenicity Analysis Set. GMFR will be presented from before vaccination to each specified post-vaccination time point.

Also, bar charts including LLOQ and ULOQ lines and with different color codes for data from subjects according to their N-binding assessment for the respective visit, time point and line plots with mean and 95% CIs over time by Cohort and trial part will be presented for VOC and the reference strains.

The analysis described above will also be performed on Immunogenicity Analysis Per Protocol Set.

6.2.1.2 SR to reference and VOC specific NT at each specified post-vaccination time point

The number and percentage of subjects with SR, including the associated 2-sided 95% CI at each specified post-vaccination time point will be provided by Cohort based on Immunogenicity Analysis Set.

The above analyses will also be performed on immunogenicity per protocol set.

6.2.2 Immunogenicity endpoints – Part B

6.2.2.1 GMT of Reference and VOC specific NT at 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) and Dose 2 of BNT162b2

The same analysis as described in Section 6.2.1.1 will be repeated for Part B Cohorts 1 at 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) and Dose 2 of BNT162b2 for subjects from the Phase III trial BNT162-02 / C4591001.

6.2.2.2 SR of Reference and VOC specific NT at 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) and Dose 2 of BNT162b2

The same analysis as described in Section 6.2.1.2 will be repeated for Part B Cohorts 1 at 1 month after one dose of BNT162b2 (B.1.1.7 + B.1.617.2) and Dose 2 of BNT162b2 for subjects from the Phase III trial BNT162-02 / C4591001.

6.2.2.3 GMT of Reference and VOC specific NT at 1 month after one dose of BNT162b2 (B.1.617.2) and Dose 2 of BNT162b2

The same analysis as described in Section 6.2.1.1 will be repeated for Part B Cohorts 4 at 1 month after one dose of BNT162b2 (B.1.617.2) and Dose 2 of BNT162b2 for subjects from the Phase III trial BNT162-02 / C4591001.

6.2.2.4 SR of Reference and VOC specific NT at 1 month after one dose of BNT162b2 (B.1.617.2) and Dose 2 of BNT162b2

The same analysis as described in Section 6.2.1.2 will be repeated for Part B Cohorts 4 at 1 month after one dose of BNT162b2 (B.1.617.2) and Dose 2 of BNT162b2 for subjects from the Phase III trial BNT162-02 / C4591001.

6.2.2.5 GMT of Reference and VOC specific NT at 1 month after Dose 2 and Dose 3 of BNT162b2 (B.1.1.7 + B.1.617.2) for Cohort 6 in COVID-19 vaccine-naïve subjects

The same analysis as described in Section 6.2.1.1 will be repeated for Part B Cohorts 6 at 1 month after Dose 2 and Dose 3.

6.2.2.6 SR of Reference and VOC specific NT at 1 month after Dose 2 and Dose 3 of BNT162b2 (B.1.1.7 + B.1.617.2) for Cohort 6 in COVID-19 vaccine-naïve subjects

The same analysis as described in Section 6.2.1.2 will be repeated for Part B Cohort 6 at 1 month after Dose 2 and Dose 3.

6.2.2.7 GMTs and GMRs of VOC specific NTs at 3 weeks after 1 dose BNT162b2 (B.1.1.7 + B1.617.2) in COVID-19 vaccine-naïve subjects vs VOC specific NTs at 1 month after one booster dose of BNT162b2 (B.1.1.7 + B1.617.2) in BNT162b2-experienced subjects (Cohort 6 with prior infection vs Cohort 1 without prior infection)

The same analysis as described in Section 6.1.2.5 will be repeated for Part B Cohort 6 vs Cohort 1 comparison.

6.2.2.8 SRs and differences in SRs of VOC specific NT at 3 weeks after 1 dose of BNT162b2 (B.1.1.7 + B1.617.2) in COVID-19 vaccine-naïve subjects vs VOC specific NTs at 1 month after one booster dose of BNT162b2 (B.1.1.7 + B1.617.2) in BNT162b2-experienced subjects (Cohort 6 with prior infection vs Cohort 1 without prior infection)

The same analysis as described in Section 6.1.2.6 will be repeated for Part B Cohort 6 at vs Cohort 1 comparison.

6.2.2.9 GMTs and GMRs of VOC specific NTs at 3 weeks after 1 dose of BNT162b2 (B.1.1.7 + B1.617.2) in COVID-19 vaccine-naïve subjects with prior infection vs VOC specific NTs at 1 month after 2 doses of BNT162b2 (B.1.1.7 + B1.617.2) in COVID-19 vaccine-naive subjects without prior infection (Cohort 6 with prior infection vs Cohort 6 without prior infection)

The same analysis as described in Section 6.1.2.5 will be repeated for Part B Cohort 6 with prior infection vs Cohort 6 without prior infection.

6.2.2.10 SRs and differences in SRs of VOC specific NT at 3 weeks after 1 dose of BNT162b2 (B.1.1.7 + B1.617.2) in COVID-19 vaccine-naïve subjects with prior infection vs VOC specific NTs at 1 month after 2 doses of BNT162b2 (B.1.1.7 + B1.617.2) in COVID-19 vaccine-naive subjects without prior infection (Cohort 6 with prior infection vs Cohort 6 without prior infection)

The same analysis as described in Section 6.1.2.6 will be repeated for Part B Cohort 6 with prior infection vs Cohort 6 without prior infection.

6.2.3 Immunogenicity endpoints – Part C

6.2.3.1 GMT of VOC specific NT at baseline and at each post-vaccination time point for Cohorts 7, 8 and 9

The same analysis as described in Section 6.2.1.1 will be repeated for Part C Cohorts 7, 8 and 9.

A bar chart including LLOQ and ULOQ lines and with different color codes for data from subjects according to their N-binding assessment for the respective visit, time point and line plot of GMTs and the associated 2-sided 95% CIs will be presented at each post-vaccination time point for each cohort by trial part.

The analysis will be performed on Immunogenicity Analysis Per Protocol Set as well. The number of subjects with valid and determinate assay results for the specified assay at each post-vaccination time point and a summary of GMT and its associated 2-sided 95% CIs will be presented by cohort.

6.3 Exploratory endpoints

6.3.1 Exploratory – Part A

The exploratory analyses not specified in Section 6.3 are out of scope of this SAP and may be described in a different document.

6.3.1.1 CD4+ and CD8+ T-cell responses (Ex vivo ELISpot)

Only descriptive analyses will be performed and no formal hypotheses will be tested for these exploratory endpoints.

In the clinical trial report, only fitness-normalized T-cell response data (normalized background is subtracted from the normalized experiment spot count to account for varying background when comparing samples) will be analyzed and all spot counts are extrapolated to number of spots per 10^6 cells.

All analyses on Ex vivo ELISpot data will be performed on the Immunogenicity Analysis Set. If applicable, analyses may be performed on the Immunogenicity Analysis Per Protocol Set as well.

Visits for blood draws for T-cell responses with visit windows can be found in Table 7. Window per CTP and additional grace period will be applied for the respective analysis set in the table.

Imputation and missing values:

Missing values for Ex vivo ELISpot data will not be imputed. Ex vivo ELISpot data outside the respective sampling windows applicable for the analysis in Table 7 will not be included in the analyses. No limit of detection applies to this endpoint.

If expert call responses for the combined target "spike protein pools 1 + 2" are not derived in the source data, they will be derived as follows:

- positive response: if at least one of spike protein pool 1 and spike protein pool 2 has a positive response
- no response: if both spike protein pool 1 and spike protein pool 2 have no response
- not evaluable: otherwise (if both spike protein pool 1 and spike protein pool 2 are not evaluable, if one of the spike protein pools has no response and the other is not evaluable).

Analysis:

ELISpot results per sample and subject response evaluation results will be reported separately for each T-cell type (CD4+ T-cells, CD8+ T-cells) and for each variant.

Spot counts will be summarized with Mean, SD, Median, Q1, Q3, Min and Max by cohort (in part A: Cohorts 1-6 separately), variant (FL-S, FLS B.1.1.7, FLS B.1.617.2), target (spike protein pool 1, spike protein pool 2, sum of spike protein poor 1 + 2), and sampling time point (visit relative to dose: Day 1, Day 8, Day 29, Day 180, Day 360) if there is data available. Moreover, responses (expert calls: positive response, no response, not evaluable) will be summarized in the same manner for all applicable sampling time points.

Scatterplots for IFNg spot assessments over time will be produced comparing cohorts per variant and target. Moreover, boxplots (with min, max, median, quartiles) for Day 8 and Day 29 will be produced comparing cohorts per variant and target.

Spot count and expert call response data used in the analysis will be listed per subject for all assessments in the Immunogenicity Analysis Set.

All tables, figures and listings for this endpoint will be produced separately for CD4+- and for CD8+- T-cells.

6.3.2 Exploratory – Part B

6.3.2.1 GMT, GMFR and SR of Reference and VOC specific NT at each post vaccination time point for Cohort 1 and 4 in BNT162b2-experienced subjects

The same analysis as described in Section 6.2.1.1 will be repeated for GMT and GMFR at each post-vaccination time point.

The same analysis as described in Section 6.2.1.2 will be repeated for SR at each post-vaccination time point.

6.3.2.2 GMT, GMFR and SRs of Reference and VOC specific NT at each post vaccination time point for Cohort 6 in COVID-19 vaccine-naïve subjects

The same analysis as described in Section 6.2.1.1 will be repeated for GMT and GMFR at each post-vaccination time point.

The same analysis as described in Section 6.2.1.2 will be repeated for SR at each post-vaccination time point.

6.3.2.3 Number of confirmed COVID-19 cases

The number and percentage of subjects reporting a confirmed COVID-19 case during the surveillance period will be presented by Cohort. In addition, the incidence of confirmed COVID-19 cases per 1,000 person-years of follow-up will also be presented.

6.3.2.4 Nucleocapsid-binding antibody seroconversion

The same analysis as described in Section 6.3.2.3 will be repeated for the incidence of N-binding antibody seroconversion.

6.3.3 Exploratory – Part C

6.3.3.1 GMT, GMFR and SR of Reference and VOC specific NT at baseline and post-vaccination time point for Cohorts 7, 8 and 9

The same analysis as described in Section 6.2.1.1 will be repeated for GMT and GMFR at baseline and post-vaccination time point for Cohort 7, 8 and 9.

The same analysis as described in Section 6.2.1.2 will be repeated for SR at baseline and post-vaccination time point for Cohort 7, 8 and 9.

6.3.3.2 Number of confirmed COVID-19 cases

The same analysis as described in Section 6.3.2.3 will be conducted for all Part C Cohorts.

6.4 Subgroup analysis

For Part B and Part C, subgroup analyses based on age group (18 to 55 years, 56 to 85 years, 18 to 64 years, and 65 to 85 years), comorbidities which are relevant for COVID-19, i.e., obesity (BMI <30 kg/m^2 and BMI ≥ 30 kg/m^2), and diabetes mellitus (yes and no) will be performed for immunogenicity endpoints and primary safety endpoints.

6.5 Other summaries and analyses

The conversion rule for days to month and days to year, the analysis visit, the definition of baseline and the computation of trial day are as defined in Section 3.4.

6.5.1 Baseline and demographic characteristics

6.5.1.1 Demographic characteristics

Demographic characteristics, including age (years) at screening and age group (18 to 55 years, 56 to 85 years, 18 to 64 years, and 65 to 85 years), sex, childbearing potential (yes or no), ethnicity, race, country, height(cm), body weight(kg), Body Mass Index (BMI [kg/m^2]

Underweight (below 18.5), Normal weight (18.5 to 24.9), Over weight (25 to 29.9), Obese (30 and above)), diabetes mellitus (yes or no), baseline SARS-CoV-2 infection status (Positive or Negative), and Time from the second dose of BNT162b2 (received prior to the trial) to the first trial vaccination (days) will be summarized for each cohort and trial part based on Safety Set, Immunogenicity Analysis Set, and all randomized subjects for Part C. For Part C, type of test performed, when the last SARS-CoV-2 infection prior to the IMP dose was diagnosed (time between prior SARS-CoV-2 infection and trial randomization), whether infection with SARS-CoV-2 Omicron variant confirmed, number of prior COVID-19 RNA vaccines and time from the last dose of COVID-19 RNA vaccination (received prior to the trial) to trial randomization will be presented by Cohort. Summary for the control group subjects from the Phase III trial BNT162-02 / C4591001 will also be included.

6.5.1.2 Medical history

Each reported medical history term will be mapped to a system organ class (SOC) and preferred term (PT) according to MedDRA. The number and percentage of subjects having at least 1 diagnosis, overall and at each SOC and PT level, will be summarized for the Safety Set and all randomized subjects for Part C by Cohort and trial part.

6.5.2 Trial conducts and subject disposition

6.5.2.1 Subject disposition

The numbers and percentages of subjects on Screened Set who received vaccinations (IMP Doses 1, 2 and 3, as applicable for each cohort), who completed treatment and completed the trial will be tabulated by cohort. In addition, the number and percentage of subjects who discontinued treatment early and/or withdrew from the trial early along with the primary reasons for treatment discontinuation, withdrawn after last IMP dose and before 1 month post- last IMP dose visit, withdrawn after 1 month post last IMP dose visit, will be tabulated by cohort. The primary reasons for trial discontinuation will be those as specified in the database. Subjects in Cohort 2 who received two IMP doses and in Cohort 6 who received three IMP doses will be considered as treatment completer.

Subjects excluded from each analysis set will also be summarized separately along with the reasons for exclusion by cohort and trial part. Summary for the control group subjects from the Phase III trial BNT162-02 / C4591001 will also be included.

6.5.2.2 Protocol deviation

The number and percentage of subjects in the Screened Set with Important protocol deviation (IPD) will be presented by cohort and trial parts, for each protocol deviation category/sub-category. Also, a listing of all protocol deviations (PDs) will be presented.

6.5.2.3 Blood samples for assay

The number and percentage of subjects' blood sample drawn for immunogenicity will be tabulated for the Safety set and all randomized subjects for Part C by cohort and trial Part. A listing of subjects providing blood samples within and outside of the protocol-specified time frames will be provided. Summary for the control group subjects from the Phase III trial BNT162-02 / C4591001 will also be included.

6.5.2.4 E-diaries

E-diary transmission will be summarized by cohort and trial part using the Safety Set. A listing of subjects who were vaccinated and completed e-diaries after each dose will be provided.

6.5.3 IMP exposure

For each dose, the number and percentage of subjects receiving each trial intervention will be tabulated for each cohort and trial part.

6.5.4 Prior/Concomitant medications and concomitant procedures

Prior and Concomitant medications (CM) will be summarized, separately, by Anatomic Therapeutic Chemical (ATC) classification level 4 and preferred name for the Safety Set and all randomized subjects for Part C by cohort and trial part. The number and percentage of subjects receiving each medication after Dose 1 will be tabulated by cohort and trial part.

Concomitant medications administered due to local reactions and systemic events during time interval Day 1 to 7 days post each dose will be summarized. Bar chart with the number and percentage of subjects with any CM for local reactions and systemic events.

Concomitant procedures (including non-drug therapies) will be listed only.

6.6 Other safety analyses

Local reaction, systemic event, AE, and SAE summaries and analyses are described under Primary Endpoints is Section 6.1.1.

6.6.1 Laboratory assessments

Safety laboratory parameters including hematology, clinical chemistry analysis, and urinalysis will be summarized at screening using descriptive summary statistics for each parameter by cohort and trial part for safety set.

Abnormal and clinically significant, abnormal and not clinically significant, and normal laboratory values will be summarized for each parameter at baseline by cohort and trial part.

Laboratory results will be listed along with the reference ranges and values below or above the reference ranges will be flagged.

6.6.2 Vital signs and physical examinations

Vital sign parameters to be summarized using descriptive statistics for heart rate, systolic blood pressure and diastolic blood pressure. Absolute values and change from baseline for oral temperature will be summarized by cohort and visit for each trial part.

Absolute values and change from baseline for each ECG parameters will be summarized by cohort, and visit for each trial part. Baseline is the last non-missing measurement or sample collected prior to first IMP injection.

Abnormal clinically significant, abnormal not clinically significant, and normal values of ECG assessment and heart rate will be summarized by visit, Cohort and trial part. Number

of subjects with missing measurements for post dose visits will be computed as number of subjects who received the specific dose minus the number of subjects with non-missing values at each visit.

Physical examinations including body weight will be performed and clinically significant abnormalities will be summarized by cohort and trial part. At a minimum, assessments of cardiovascular, respiratory, gastrointestinal, and neurological systems will be performed.

7 ANALYSES TIMING

7.1 Interim analyses and summaries

No formal interim analysis will be performed. However, the safety and immunogenicity analysis with data up to 1 month post dose for Part A (Cohort 1, 3, 4 and 5), 1 month post Dose 2 for Part A (Cohort 2 and 6) will be performed.

7.2 Safety Review Committee

This trial will use an SRC. The SRC will at least comprise a sponsor medical representative, the Medical Monitor, an independent medical physician, an independent cardiologist, a statistician, coordinating investigator, a sponsor-independent investigator, and on an *ad hoc* basis, a representative for the respective trial site depending on the trial subject in question.

Safety reviews of the data will be conducted by the SRC approximately every 4 weeks and on an *ad hoc* basis. The SRC will review all safety data, including AESIs, and SAEs, as well as laboratory data and other relevant safety data.

After all subjects enrolled into Part A, Part B and Part C have completed their 1 month follow-up visit, the SRC members will continue to receive safety data (related AEs, AESIs, SAEs) on a monthly basis, but further SRC meetings will occur approximately every 6 months unless triggered earlier by SRC member requests or if suspected unexpected serious adverse reactions (SUSARs)/AESIs are reported.

All outputs required and data cut off requirements will be documented prior to each SRC. More details can be found in latest SRC charter.

8 CHANGES IN STATISTICAL ANALYSIS FROM PROTOCOL

8.1 Additional analysis sets for safefy and immunogenicity analysis

In Table 8 of Section 4, All Randomized Subjects was added for demographic and other baseline data analysis in Part C. Immunogenicity Analysis Per Protocol Set and All-available Immunogenicity Set (mITT) were added for additional immunogenicity analysis in Part B and C immunogenicity.

8.2 Changes in interim analysis

the safety and immunogenicity analysis with data up to 1 month post dose for Part A (Cohort 1, 3, 4 and 5), 1 month post Dose 2 for Part A (Cohort 2 and 6) will be performed. The primary immunogenicity analysis for all Part B cohorts and for Part C Cohorts 7 and 8 will be performed in the final analysis for clinical study report.

8.3 Withdrawal of primary objectives to demonstrate the non-inferiority of immune response against VOC (B.1.1.7 and B.1.617.2) after two doses of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in terms of GMT and SR for COVID-19 vaccine-naïve subjects in Part B Cohort 6

In COVID-19 vaccine naïve individuals (recruited into cohort B6), the original primary immunogenicity objectives were to demonstrate the non-inferiority of immune response against Variants of Concern (B.1.1.7 and B.1.617.2) after two doses of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) to the immune response after two doses of original BNT162b2 in subjects without evidence of infection from the Phase I/ II/ III trial BNT162-02/ C4591001 in terms of geometric mean titer (GMT) and seroresponse rate (SR).

By the time the cohort had been fully recruited in August 2022, approximately 75% participants recruited into cohort B6 – required to be without known history of SARS-CoV-2 infection, self-reported at the screening visit according to the exclusion criteria - retrospectively turned out to be seropositive for SARS-CoV-2 by planned N-binding antibody assessment at baseline. The cohort hence was COVID-19 vaccine-naive, but not SARS-CoV-2-naïve as originally foreseen.

In BNT162-17 protocol version v7.0, new primary and secondary immunogenicity objectives for COVID-19 vaccine-naïve subjects in Part B were introduced aiming to support FDA authorization of a single-dose primary regimen of the 2023/2024 season variant-adapted BNT162b2 vaccine targeting XBB.1.5 in previously unvaccinated individuals with and without previous SARS-CoV-2 infections. The new primary immunogenicity objectives were thus to demonstrate the non-inferiority of immune response against reference strain after one dose of multivalent vaccine BNT162b2 (B.1.1.7 + B.1.617.2) in COVID-19 vaccine-naïve subjects with evidence of prior infection (cohort B6 of BNT162-17) to the immune response after two doses of original BNT162b2 in subjects without evidence of infection from the Phase I/ II/ III trial BNT162-02/ C4591001 in terms of GMT and SR.

The original objectives in cohorts B1 and B4, the new primary immunogenicity objective in cohort B6, the primary safety objectives and all secondary objectives in cohort B6 will be analysed according to the clinical trial protocol (CTP).

However, since the population recruited into cohort B6 (unvaccinated but not SARS-CoV-2 naïve) did not reflect the originally foreseen population for this cohort, it was decided that the data analysis for the original primary immunogenicity endpoints for cohort B6 would not be performed according to the CTP. Concretely that entails the following:

1. While Variants of Concern (VOC) and reference strain Virus Neutralization Tests (VNTs) will be determined for all samples taken in BNT162-17 cohort B6, the data will not be used for the primary endpoint. The data will be used to address secondary and exploratory endpoints only.

2. The control cohort from the pivotal study BNT162-02, in which two doses of BNT162b2 were assessed in previously unvaccinated individuals, will not be picked and no data will be generated.

3. An amendment to the CTP was no longer possible because the study had been closed in one of the four countries it was performed in and global end of trial was achieved on 04 October 2023. The above approach will therefore be described in the statistical analysis plan (SAP) and clinical study report (CSR).

9 REFERENCES

Agresti A. Introduction: distributions and inference for categorical data. In: Agresti A, ed. *Categorical data analysis*. 2nd ed. Hoboken, NJ: John Wiley & Sons; 2002:1-35.

Miettinen O, Nurminen M. Comparative analysis of two rates. *Stat Med*. 1985;4(2):213-226. doi:10.1002/sim.4780040211.

FDA Guidance 2007. FDA Guidance for Industry. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

BNT162-02 / C4591001 (NCT04368728). Trial to describe the safety, tolerability, immunogenicity, and efficacy of RNA vaccine candidates against COVID-19 in healthy individuals. BioNTech clinical trial was completed.

10 APPENDICES

Appendix 1. List of abbreviations

Abbreviation	Term
AE	Adverse event
AESI	Adverse event of special interest
BNT162b2 (B.1.1.529)	Monovalent vaccine specific for SARS-CoV-2 Omicron subvariant BA.1, i.e., B.1.1.529.1
BNT162b2 (B.1.1.7)	Monovalent vaccine specific for SARS-CoV-2 Alpha variant
BNT162b2 (B1.617.2)	Monovalent vaccine specific for SARS-CoV-2 Delta variant
CI	Confidence interval
CD	Cluster of differentiation, e.g., CD4, CD8, glycoproteins that serves as a co-receptor for T-cell receptors
COVID-19	Coronavirus disease 2019
CMI	Cell-mediated immune
CRF	Case report form
e-diary	Electronic diary
EDC	Electronic Data Capture (system)
GMC	Geometric mean concentration
GMFR	Geometric mean fold rise
GMR	Geometric mean ratio
GMT	Geometric mean titer
IgG	Immunoglobulin G
IMP	Investigational Medicinal Product
LLOQ	Lower limit of quantitation
MedDRA	Medical Dictionary for Regulatory Activities
MH	Medical history
N/A	Not applicable
NAAT	Nucleic acid amplification-based test
NT	Neutralizing titer
PT	Preferred term
RBD	Receptor binding domain
RNA	Ribonucleic acid
S	Spike protein
S1	Spike protein S1 subunit
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOC	System organ class
SP1	Spike protein pool 1
SP2	Spike protein pool 2
SR	Seroresponse
SRC	Safety Review Committee
TEAE	Treatment-emergent adverse event
VOC	Variant of concern

Appendix 2. Subject selection for primary and secondary immunogenicity analysis in Part B

To perform the primary immunogenicity analyses specified in Section 6.1.2.5 and 6.1.2.6 and the secondary immunogenicity analyses specified in Section 6.2.2.7, 6.2.2.8, 6.2.2.9, and 6.2.2.10, subjects in trial BNT162-17 and the control group subjects in Phase III trial BNT162 02 / C4591001 were selected with the following criteria:

- *COVID-19 vaccine-naïve subjects with evidence of prior SARS-CoV-2 infection in Part B Cohort 6* who had positive test results (n-capsid and NAAT test) at baseline; did not have key protocol deviations that lead to exclusion from immunogenicity analysis; immunogenicity samples at 3-week post Dose 1 were collected within grace period window (16 to 26 days from Dose 1); and had sufficient volume of serum samples were eligible for the analysis.

  For comparison between subjects in Cohort B1 without evidence of infection and subjects in Cohort B6 with prior infection, an equivalent number of subjects were selected from B6 positive group randomly to match number of B1 negative subjects. These subjects are randomly selected to support the Variants Neutralizing Titers analysis.

- COVID-19 vaccine-naïve subjects without evidence of SARS-CoV-2 infection in Part B Cohort 6 who had negative n-capsid test result at baseline and 1-month post Dose 2; negative local and central NAAT test result from baseline to 1-month post Dose 2; no COVID-19 infection AE events; did not have key protocol deviations that lead to exclusion from immunogenicity analysis; immunogenicity samples at 1-month post Dose 2 were collected within grace period window (19 to 39 days from Dose 2); and had sufficient volume of serum samples were eligible for the analysis.
- COVID-19 vaccine-experienced subjects with one booster dose of BNT162b2 (B.1.1.7 + B1.617.2) without evidence of SARS-CoV-2 infection in Part B Cohort 1 who had negative n-capsid test result at baseline and 1-month post Dose 1; did not have key protocol deviations that lead to exclusion from immunogenicity analysis; immunogenicity samples at 1-month post Dose 1 were collected within grace period window (19 to 39 days from Dose 1); and had sufficient volume of serum samples were eligible for the analysis.
- Subjects without evidence of SARS-CoV-2 infection in Pfizer Phase III trial BNT162-02 / C4591001
  - had sufficient blood volume at baseline and 1-month post dose 1 (total volume >1000 unit
  - had no evidence of infection up to 1-month post Dose 2
  - enrolled in Phase 3 part of the trial and randomized to BNT162b2 30 mcg group and received 2 doses of BNT162b2 30 mcg
  - HIV negative and not in multiple trials
  - did not have important PDs
  - did not violate inclusion criteria
  - had 1MPD2 (V3) blood draw within 28-42 days after Dose 2

- were not enrolled in BNT162-17 trial.

After applying above conditions, there were ~20,000 subjects eligible. Subjects were selected randomly with matching age and sex with each of subjects in Part B Cohort 6 of BNT162b2-17 (~276 total) using SAS Proc Surveyselect procedure.

To perform the primary analyses specified in Section 6.1.2.1, 6.1.2.2, 6.1.2.3, and 6.1.2.4, subjects in trial BNT162-17 and the control group subjects in Phase III trial BNT162 02 / C4591001 were selected with the following criteria:

- BNT162b2 vaccine-experienced subjects with one booster dose of BNT162b2 (B.1.1.7 + B1.617.2) in Part B Cohort 1 and with one booster dose of BNT162b2 (B1.617.2) in Part B Cohort 4 who had valid samples at baseline and 1-month post Dose 1 were eligible for the analysis.
- Subjects in Pfizer Phase III trial BNT162 02 / C4591001
  - were randomized originally to BNT162b2 Phase 2/3 (30 mcg)
  - had two doses at vaccination 1 and 2 as randomized
  - were not enrolled in BNT162-17 trial
  - sample volume at baseline (V1) and 1 month post 2nd dose (V3) are >500 unit
  - enrolled in phase 3 study, HIV negative, and not in multiple trials
  - not selected for analysis specified in Section 6.1.2.5 and 6.1.2.6 and the secondary immunogenicity analyses in Section 6.2.2.7, 6.2.2.8, 6.2.2.9.

After applying above conditions, there were ~20,000 subjects from Pfizer Phase III trial BNT162 02 / C4591001_eligible. Subjects were selected randomly with matching age and sex with each of eligible subjects in Part B Cohort 1 and Cohort 4 of BNT162b2-17 (~700 total) using SAS Proc Surveyselect procedure.