CLINICAL TRIAL PROTOCOL
Trial number: BNT165-02

Document version:	8.0	**Date:**	04 FEB 2025
Sponsor name:	BioNTech SE		
Sponsor address:	An der Goldgrube 12, 55131 Mainz, Germany		

Trial title:	A randomized, dose-escalation Phase I/IIa trial with controlled human malaria infection to evaluate safety, tolerability, immunogenicity and efficacy of an investigational RNA-based vaccine for prevention of *P. falciparum* malaria in healthy malaria-naïve adults
Brief lay title:	A clinical trial to evaluate the safety, efficacy and immune responses after vaccination with an investigational RNA-based vaccine against malaria
Trial phase:	Phase I/IIa
Indication:	Active immunization against *Plasmodium falciparum* (*P. falciparum*) malaria
Investigational medicinal product (IMP):	BNT165e; a combination of BNT165c and BNT165d (composed of BNT165d1 and BNT165d2)
Sponsor signatory:	Dr Thierry Rolling Director, Clinical Development Infectious Diseases, BioNTech SE
Regulatory identifiers:	National Clinical Trial (NCT) number: NCT06069544; Investigational New Drug application (IND) number: 29905
Trial sites:	Sites are planned in the United States (US)
Trial medical monitor:	For the name and contact details, see the Trial Contact List filed in the Investigator's Site File (ISF)

The "sponsor signatory" is person authorized to sign (approve) the protocol and any protocol amendment(s) for the sponsor. A list of key sponsor personnel involved in the preparation of this protocol and the conduct of the trial, including their full names, titles, roles, and responsibilities, will be maintained. These personnel include the sponsor's medical expert for the trial. Some sponsor tasks in the conduct of this trial may be delegated, e.g., to contract research organization (CRO) staff. Documentation of any delegation of responsibilities will be maintained.

Statement of compliance: This trial will be conducted according to this protocol, the ethical principles that have their origin in the Declaration of Helsinki, ICH Good Clinical Practice (GCP), and applicable regulatory requirements.

Document history	Date	Version number
First sponsor approved version	23 JUN 2023	1.0
Second sponsor approved version	11 OCT 2023	2.0
Third sponsor approved version	17 OCT 2023	3.0
Fourth sponsor approved version	21 NOV 2023	4.0
Fifth sponsor approved version	09 JAN 2024	5.0
Sixth sponsor approved version	16 MAY 2024	6.0
Seventh sponsor approved version	15 AUG 2024	7.0
Eighth sponsor approved version	04 FEB 2025	8.0

See Section 10.9 for rationales/summaries of all protocol updates.

1 PROTOCOL SUMMARY

1.1 Synopsis

Trial title

A randomized, dose-escalation, Phase I/IIa trial with controlled human malaria infection (CHMI) to evaluate safety, tolerability, immunogenicity and efficacy of an investigational RNA-based vaccine for prevention of *P. falciparum* malaria in healthy malaria-naïve adults.

Trial rationale

The World Health Organization (WHO) estimated that there were ~249 million cases of malaria and 608,000 malaria-associated deaths in 2022, the majority of which were caused by the parasite *P. falciparum* (WHO Malaria Report 2023). The WHO is aiming to reduce the incidence and mortality due to malaria by 90% by 2030. Despite programmatic efforts, recent gains to decrease malaria morbidity and mortality have been partially reversed, underscoring the need for effective malaria vaccines and other new tools to prevent malaria and achieve the WHO goals.

BioNTech aims to develop a highly efficacious *P. falciparum* blood stage infection prevention vaccine that at an individual level prevents disease and death. When this vaccine is applied to a large enough proportion of the target population constituting the infectious reservoir, it may prevent onward transmission, and thus enable malaria elimination. The vaccine under development is intended to induce poly-specific humoral and cellular immune responses against multiple *P. falciparum* antigens expressed in different stages of its life cycle rather than relying on targeting sporozoites alone, as done by Mosquirix™ and R21/Matrix-M.

For the development of this malaria vaccine BioNTech will use the RNA technology platform used for the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) vaccine BNT162b2 "COMIRNATY®" which has been licensed for emergency use or been given conditional or full marketing authorizations in more than 100 countries globally. The lipid nanoparticle (LNP) carrier system, the chemistry (nucleoside-modified) and the sequence design of the RNA molecule's untranslated backbone elements (cap, untranslated regions, polyA tail) will remain unchanged. Only the encoded open reading frames will change and will represent *P. falciparum* derived antigens. Also, the same well characterized manufacturing process, controls and possibly also manufacturing facilities, used for BNT162b2 will be utilized, as well as the same route of administration.

BioNTech has started clinical development of a malaria vaccine with the trial BNT165-01 that assesses safety, reactogenicity, and immunogenicity of BNT165b1, an RNA encoding part of the *P. falciparum* circumsporozoite protein (*Pf*CSP) (NCT05581641).

The malaria vaccine candidate BNT165e tested in this clinical trial is a combination of three distinct RNAs, BNT165c and BNT165d (composed of BNT165d1 and BNT165d2), encoding *P. falciparum* antigens each encapsulated in the LNP. BNT165d1 and BNT165d2 both encode conserved, immunogenic segments of liver-stage expressed proteins. BNT165c encodes the full *Pf*CSP. Some cohorts will assess BNT165c and BNT165d on their own to evaluate their contribution to the safety and immunogenicity profile of BNT165e. Neither BNT165c nor BNT165d are intended to be developed as individual vaccine candidates and will be used only in the context of BNT165e.

We will start the clinical development of BNT165e with this trial BNT165-02 with Part A evaluating the safety, tolerability and immunogenicity of different dose combinations of BNT165c and BNT165d. Some cohorts will assess BNT165c and BNT165d on their own to evaluate their contribution to the safety and immunogenicity profile of BNT165e. Neither BNT165c nor BNT165d are intended to be developed as individual vaccine candidates and will be used only in the context of BNT165e.

CCI [redacted]

Objectives and endpoints

OBJECTIVES	ENDPOINTS	OUTCOME MEASURES
Primary objective (Part A)		
To describe the safety and tolerability of BNT165e and its components in healthy adults.	• Solicited local reactions (pain, erythema/redness, induration/swelling). • Solicited systemic reactions (vomiting, diarrhea, headache, fatigue, muscle/joint pain, and fever). • AEs • SAEs • MAAEs	For each cohort in subjects receiving at least one dose of trial intervention: • Frequency of solicited local reactions at the injection site recorded up to 7 d after each dose. • Frequency of solicited systemic reactions recorded up to 7 d after each dose. • Frequency of subjects with at least one AE occurring until 28 d after each dose. • Frequency of subjects with at least one MAAE occurring until 24 weeks after last received IMP dose. • Frequency of subjects in each cohort with at least one SAE occurring until 24 weeks after last received IMP dose.
Secondary objective (Part A)		
To evaluate the CSP-specific humoral immune response of BNT165e and its components.	Anti-CSP IgG levels at specified timepoints in the SoA (Section 1.3).	For each cohort: • Descriptive statistics on antibody levels (including geometric means and 95% CI) at assessed timepoints.
Exploratory objective (Part A)		
To evaluate the cell mediated immune response	CD4 and CD8 T-cell responses at baseline and specified timepoints post-immunization (Section 1.3).	• Frequency of antigen-specific T cells at assessed timepoints.

Abbreviations: AE = adverse event; CD = cluster of differentiation; CI = confidence interval; CSP = circumsporozoite protein; d = day(s); IgG = immunoglobulin G; IMP = investigational medicinal product; MAAE = medically attended adverse event; SAE = serious adverse event; SoA = schedule of activities.

Overall design

Part A of this trial will evaluate the safety, tolerability, and immunogenicity of a multi-component vaccine BNT165e, consisting of three mRNA components encoding the *Pf*CSP and immunogenic segments of liver-stage expressed proteins.

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Part A

In Part A, the combination of the three components will be evaluated in different dose combinations in a 3-dose schedule to select a safe, tolerable, and immunogenic dose combination, and to assess the impact of third dose on immunogenicity. One dose combination will also be tested in a Day 1, Day 29 and Day 57 dosing schedule in Cohort 10 (Section 1.3). Part A of the trial will be observer-blind.

Up to 138 healthy subjects are divided into 10 cohorts with different dose combinations and will be randomized 5:1 active:placebo. Number of subjects may be increased up to 177 in the event of subject replacement or re-enrollment.

Table 1 denotes the dose combinations being tested in the different cohorts and the planned number of subjects to be included.

Table 1: Dose combinations (Part A)

Cohort	Dose level (µg) BNT165c	Dose level (µg) BNT165d [a]	Sample size (active:placebo) [d]
1	10	0	10:2
2	≤30	0	10:2
3	≤70	0	10:2
4	0	30	10:2
5	≤30	10	10:2
6	≤10	≤30	10:2
7	≤30	≤30	15:3
8	≤70	≤30	15:3
9	≤100	0	10:2 [b]
10 [c]	≤70	≤30	10:2

a BNT165d will be a 1:1 ratio of BNT165d1 and BNT165d2.

b The sample size for Cohort 9 may be increased to 15:3 (active:placebo) based on IRC decision.

c One of the dose levels tested in Cohorts 1 to 8 will be selected by the IRC for Cohort 10 based on all available safety data until at least 28 days after Cohorts 1 to 9 have received their first dose.

d Subject numbers may increase to replace subjects that are either discontinued from further vaccination or discontinued from the trial.

Abbreviation: IRC = Internal Review Committee.

For the planned assessments and visits, see the SoA in Section 1.3. This trial will use a staggered dose-escalation schema with sentinel subjects in Cohorts 1 to 9 (see Figure 1).

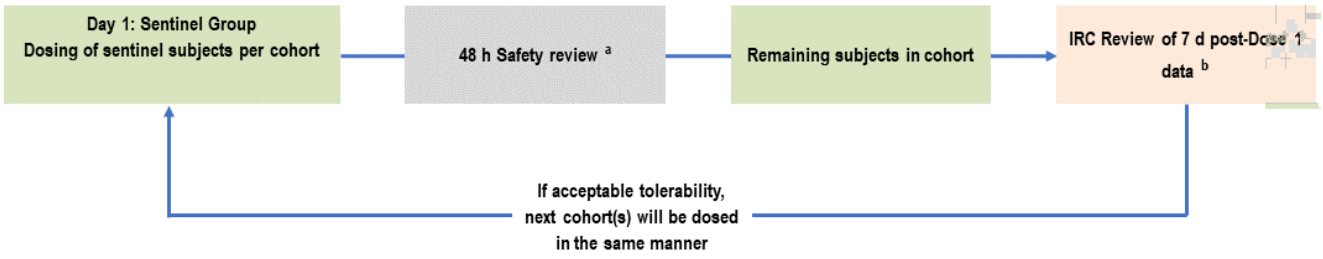

Figure 1: Staggered dosing process for Cohorts 1 to 8 (Part A)

a Further dosing of subjects will proceed if the investigator considers the vaccine reactogenicity is acceptable (only Grade 2 or lower events or after consultation with medical monitor) and no stopping/pausing rules are met.

b Further opening of new cohorts will proceed if the IRC considers the reactogenicity and safety profile of the vaccine to be acceptable and no stopping/pausing rules are met.

Abbreviations: d = day(s); h = hour(s); IRC = Internal Review Committee.

Within all cohorts in Part A, subjects will be randomized in a 5:1 ratio to IMP vs. placebo.

Cohort 1 will be initiated with two subjects as the sentinel group, whereby at least one sentinel subject will receive active IMP. Cohorts 2 to 9 will be initiated with three subjects as the sentinel group, whereby at least two sentinel subjects in each cohort will receive active IMP. The sentinel subjects in one cohort will be dosed at the same clinical trial site at least 1 hour (h) apart. The sentinels will complete 48 h of follow-up post-Dose 1. In case the investigator considers it unsafe to continue, an *ad hoc* Internal Review Committee (IRC) will be convened to decide on continuation of the cohort. If the investigator considers the vaccine reactogenicity acceptable (only Grade 2 or lower events or after consultation with medical monitor) and no stopping/pausing rules are met, the remaining subjects per cohort can be dosed. One of the dose levels tested in Cohorts 1 to 8 will subsequently be tested with a Day 1, Day 29 and Day 57 dosing schedule in Cohort 10, without sentinel dosing.

In Part A, cohorts testing BNT165c alone will be opened first. The first 3 cohorts will be opened sequentially with an IRC meeting before each dose-escalation. Cohort 1 (10 µg BNT165c) will first be opened. If the IRC decides to continue dose-escalation based on all available 7 days (d) post-Dose 1 safety data, then Cohort 2 (≤30 µg BNT165c) will be opened. Similarly, the sentinel subjects will first be dosed, followed by the rest of the cohort after 48 h of follow-up. If the decision is made to open the next cohort, then Cohort 3 (≤70 µg BNT165c) will be opened.

At this point, the IRC will convene to assess all available clinical, laboratory and other relevant data.

If no stopping/pausing rules are met and there are no other safety concerns, then Cohort 9 (≤100 µg BNT165c) will be opened. Alternatively, if the IRC decides not to open Cohort 9, then Cohort 4 will be opened next instead. If Cohort 9 is opened, then it will be filled first. Thereafter, Cohort 4 (30 µg BNT165d) will be opened. After Cohort 4 is filled, Cohort 5 (≤30 µg BNT165c and 10 µg BNT165d) will be opened. Once all subjects in Cohorts 9 (if opened), 4 and 5 have completed 7 d follow-up post-Dose 1, all available clinical, laboratory and other relevant data will be reviewed by the IRC. If the IRC considers the safety profile of the IMP to be acceptable and no stopping/pausing rules are met, dosing will be opened for Cohort 6 (≤10 µg BNT165c and ≤30 µg BNT165d). After this cohort is filled, Cohort 7 (≤30 µg BNT165c and ≤30 µg BNT165d) will be opened. After Cohort 7 is filled, Cohort 8 (≤70 µg BNT165c and ≤30 µg BNT165d) will be opened. Based on all the safety data available until at least 28 d after Cohorts 1 to 9 have received their first dose, the IRC will decide on a dose level for Cohort 10 from one of the dose levels used in Cohorts 1 to 8. Cohort 10 will subsequently be dosed with IMP on Day 1, Day 29 and Day 57.

Provided no cohort trial treatment pausing rules are met, the reactogenicity profile is acceptable and the IRC does not identify safety findings of clinical concern, subjects who received Dose 1 in each cohort will proceed to receive Dose 2 and Dose 3. If a pausing rule is met, no additional subjects will be assigned to the trial and administration of IMP for all cohorts, including administration of Dose 2 and Dose 3 for all cohorts, will be paused until the IRC has reviewed the event(s).

The IRC may recommend that the planned escalation dose combination be reduced for a given cohort. Other unplanned dose modifications, pausing (temporary halting) of trial treatment, or even discontinuation of trial treatment may be required. See Section 7 for guidance on criteria for such cases.

In addition to the IRC, the trial medical monitor and safety physician will review the clinical and safety data on an ongoing basis, interact with trial sites as needed, and assess whether any pausing rules are met. The Independent Data Monitoring Committee (IDMC) will review summary safety data of all subjects in the clinical trial on a quarterly basis according to the IDMC charter.

For a graphical depiction of the trial, see Figure 2.

Trial duration

The planned trial duration for each subject in Cohorts 1 to 9 is up to 6 weeks screening, 26 weeks vaccination phase, and 52 weeks follow-up phase, amounting to a maximum of 84 weeks.

The planned trial duration for each subject in Cohort 10 is up to 6 weeks screening, 8 weeks vaccination phase, and 52 weeks follow-up phase, amounting to a maximum of 66 weeks.

Trial duration may exceed the listed maximum time in the event of a trial pause.

Trial population

This trial will recruit healthy, malaria-naïve adult volunteers based on medical history, screening laboratory values, and other inclusion/exclusion criteria. Inclusion and exclusion criteria for Part A are described in Sections 5.1 and 5.2, respectively.

Number of trial subjects

In Part A, up to 138 subjects will be enrolled, in cohorts as defined in Table 1. Number of subjects may be increased up to 177 in the event of subject replacement or re-enrollment. The sample size for each cohort is mainly driven by a dose-escalation trial in a limited number of subjects designed for early detection of potential safety and reactogenicity events, and is considered adequate to support the trial objectives, while minimizing the number of trial subjects exposed to a new IMP.

If a subject is discontinued either from further vaccination or discontinued from the trial, the sponsor can decide to replace this subject with a new subject. Subjects will be randomized to the vacancy in the open cohort until that cohort has filled.

Trial treatments

IMP name	BNT165e, consisting of the following components: • BNT165c • BNT165d (a 1:1 mixture of BNT165d1 and BNT165d2). Isotonic NaCl solution (0.9%) will be used as placebo in Part A.
Type	Investigational
Route of administration	IM injection in the deltoid muscle of the non-dominant arm. Other injection sites may be used if necessary.
Dose levels	Part A: Refer to Table 1.
Vaccination schedules	Part A: Three (one each on Days 1, 57, and 183) injections given to Cohorts 1 to 9. Three (one each on Day 1, Day 29 and Day 57) injections given to Cohort 10.

Abbreviations: IM = intramuscular; IMP = investigational medicinal product.

Statistical analyses

No formal statistical hypotheses will be tested in this trial. All analyses are exploratory in nature. The analysis of adverse events (AEs) and local/systemic reactions will be done using descriptive statistics. Immunogenicity data will be summarized using geometric means and the corresponding 95% confidence interval.

1.2 Schema

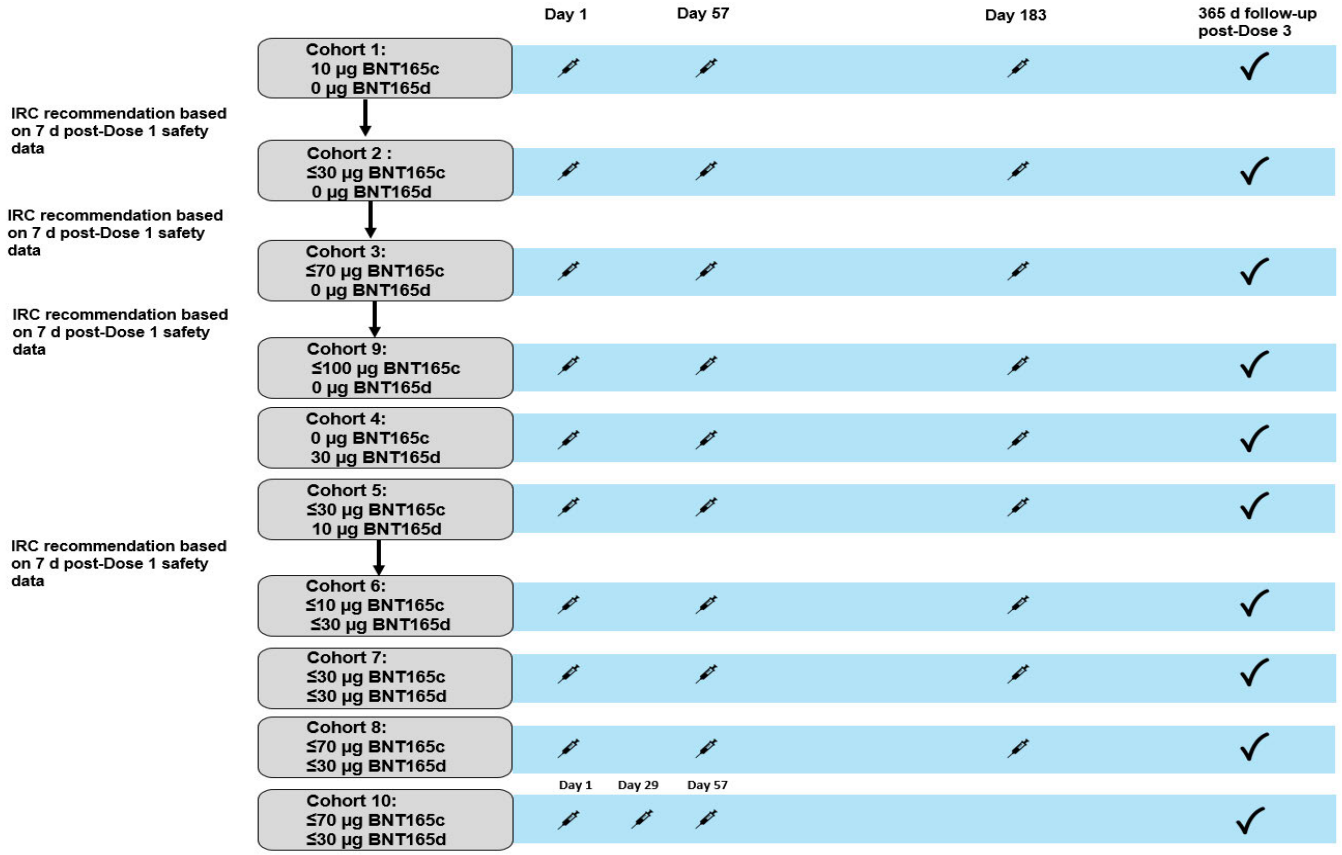

Figure 2: Graphical representation of the trial: Part A

Notes: Syringes indicate vaccination.
Abbreviations: d = day(s); IRC = Internal Review Committee.

1.3 Schedule of activities

The SoA provides an overview of the trial visits and procedures. The investigator may conduct unscheduled visits in addition to those listed in the SoA, in order to conduct evaluations or assessments required to protect the wellbeing of the trial subjects.

Table 2: Schedule of activities for three-vaccination schedule for Cohorts 1 to 9 (Part A)

Activity	**Visit (V) 0**	**V 1**	**V 2 (Call)**	**V 3 (Call)**	**V 4**	**V 5 (Call)**	**V 6 (Call)**	**V 7**	**V 8 (Call)**	**V 9 (Call)**	**V 10**	**V 11 (Call)**	**V 12**	**V 13**	**V 14 (Call)**	**V 15 (Call)**	**V 16**	**V 17 (Call)**	**V 18**	**V 19**	**V 20**	**V 21**
Visit description	**Scr**	**Dose (D) 1 Day 1**	**24 h post-D 1**	**48 h post-D 1**	**7 d post-D 1**	**14 d post-D 1**	**28 d post-D 1**	**D 2 (56 d post-D 1)**	**24 h post-D 2**	**48 h post-D 2**	**7 d post-D 2**	**14 d post-D 2**	**28 d post-D 2**	**D 3 (182 d post-D 1)**	**24 h post-D 3**	**48 h post-D 3**	**7 d post-D 3**	**14 d post-D 3**	**28 d post-D 3**	**84 d post-D 3**	**168 d post-D 3**	**365 d post-D 3 or ET**
Visit window	**up to 42 d before V 1**	**NA**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±2 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±7 d [m]**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±14 d**	**±14 d**	**±14 d**
Informed consent	X																					
Inclusion / exclusion criteria	X	X review																				
Medical history	X	X update																				
Physical exam.	X	X [a]			X [a]			X [a]			X [a]			X [a]			X [a]					
Height & body weight	X																					
Vital signs	X	X [b]			X			X [b]			X			X [b]			X					
12-lead ECG [k]	X	X			X			X			X			X			X					

Activity	**Visit (V) 0**	**V 1**	**V 2 (Call)**	**V 3 (Call)**	**V 4**	**V 5 (Call)**	**V 6 (Call)**	**V 7**	**V 8 (Call)**	**V 9 (Call)**	**V 10**	**V 11 (Call)**	**V 12**	**V 13**	**V 14 (Call)**	**V 15 (Call)**	**V 16**	**V 17 (Call)**	**V 18**	**V 19**	**V 20**	**V 21**
Visit description	**Scr**	**Dose (D) 1 Day 1**	**24 h post-D 1**	**48 h post-D 1**	**7 d post-D 1**	**14 d post-D 1**	**28 d post-D 1**	**D 2 (56 d post-D 1)**	**24 h post-D 2**	**48 h post-D 2**	**7 d post-D 2**	**14 d post-D 2**	**28 d post-D 2**	**D 3 (182 d post-D 1)**	**24 h post-D 3**	**48 h post-D 3**	**7 d post-D 3**	**14 d post-D 3**	**28 d post-D 3**	**84 d post-D 3**	**168 d post-D 3**	**365 d post-D 3 or ET**
Visit window	**up to 42 d before V 1**	**NA**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±2 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±7 d [m]**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±14 d**	**±14 d**	**±14 d**
Blood draw for viral screen [c]	13 mL																					
Blood for clinical lab. [d]	6 mL	4.5 mL [e]			4.5 mL			4.5 mL [e]			4.5 mL			4.5 mL [e]			4.5 mL					
Pregnancy test for VOCBP [f]	X	X						X						X								
Randomization to IMP or placebo		X																				
IMP or placebo injection		X						X						X								
Record pregnancies		X	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Counsel / remind subjects to use contraception	X	X			X			X			X		X	X			X		X	X		

Activity	**Visit (V) 0**	**V 1**	**V 2 (Call)**	**V 3 (Call)**	**V 4**	**V 5 (Call)**	**V 6 (Call)**	**V 7**	**V 8 (Call)**	**V 9 (Call)**	**V 10**	**V 11 (Call)**	**V 12**	**V 13**	**V 14 (Call)**	**V 15 (Call)**	**V 16**	**V 17 (Call)**	**V 18**	**V 19**	**V 20**	**V 21**
Visit description	**Scr**	**Dose (D) 1 Day 1**	**24 h post-D 1**	**48 h post-D 1**	**7 d post-D 1**	**14 d post-D 1**	**28 d post-D 1**	**D 2 (56 d post-D 1)**	**24 h post-D 2**	**48 h post-D 2**	**7 d post-D 2**	**14 d post-D 2**	**28 d post-D 2**	**D 3 (182 d post-D 1)**	**24 h post-D 3**	**48 h post-D 3**	**7 d post-D 3**	**14 d post-D 3**	**28 d post-D 3**	**84 d post-D 3**	**168 d post-D 3**	**365 d post-D 3 or ET**
Visit window	**up to 42 d before V 1**	**NA**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±2 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±7 d [m]**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±14 d**	**±14 d**	**±14 d**
Prior medications and vaccinations	X	X update																				
Record concomitant medications and vaccinations since last visit		X			X			X			X		X	X			X		X	X	X	X
Blood for humoral response assessments		30 mL [e]						30 mL [e]			30 mL		30 mL	30 mL [e]			30 mL		30 mL	30 mL	30 mL	30 mL
Blood for CMI assessment		100 mL [e]						70 mL [e]			100 mL		70 mL	70 mL [e]			100 mL		70 mL	70 mL	70 mL	70 mL [e]
Blood for HLA typing		4 mL [e]																				
Subject hotline availability	Start	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Issue, train and collect subject e-diaries		Issue, train						Re-train						Re-train			Collect					

Activity	**Visit (V) 0**	**V 1**	**V 2 (Call)**	**V 3 (Call)**	**V 4**	**V 5 (Call)**	**V 6 (Call)**	**V 7**	**V 8 (Call)**	**V 9 (Call)**	**V 10**	**V 11 (Call)**	**V 12**	**V 13**	**V 14 (Call)**	**V 15 (Call)**	**V 16**	**V 17 (Call)**	**V 18**	**V 19**	**V 20**	**V 21**
Visit description	**Scr**	**Dose (D) 1 Day 1**	**24 h post-D 1**	**48 h post-D 1**	**7 d post-D 1**	**14 d post-D 1**	**28 d post-D 1**	**D 2 (56 d post-D 1)**	**24 h post-D 2**	**48 h post-D 2**	**7 d post-D 2**	**14 d post-D 2**	**28 d post-D 2**	**D 3 (182 d post-D 1)**	**24 h post-D 3**	**48 h post-D 3**	**7 d post-D 3**	**14 d post-D 3**	**28 d post-D 3**	**84 d post-D 3**	**168 d post-D 3**	**365 d post-D 3 or ET**
Visit window	**up to 42 d before V 1**	**NA**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±2 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±7 d [m]**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**±4 d**	**±14 d**	**±14 d**	**±14 d**
Subjects report reactogenicity daily for 7 d using an e-diary		Start	=>	=>	End			Start	=>	=>	End			Start	=>	=>	End					
Investigators review e-diary data daily		Start	=>	=>	End			Start	=>	=>	End			Start	=>	=>	End					
Record AEs, MAAEs, and SAEs	X [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X [l]	X [l]	X [l]
Subject phone call		X [h]	X	X		X [j]	X	X [h]	X	X		X [j]		X [h]	X	X		X [j]				
Investigator local reaction assessment		X [i]						X [i]						X [i]								
Blood volume at visit (mL)	**19**	**138.5**			**4.5**			**104.5**			**134.5**		**100**	**104.5**			**134.5**		**100**	**100**	**100**	**100**

a Brief (symptom-targeted) physical examination, if clinically indicated. If the subject has no symptoms and a physical examination is not clinically indicated, it is not required. Further details are provided in Section 8.3.1.

b On dosing days to be taken within 1 h pre-dose and at 1 h (±15 minutes) post-dose. Further details are provided in Section 8.3.2.

c Viral screening details are provided in Section 8.3.5.

d Clinical laboratory tests details are provided in Section 8.3.4.
e On days with dosing, blood draws should be done pre-dose.
f VOCBP: The serum β-HCG pregnancy test at Visit 0 is performed using the sample collected for clinical laboratory tests. Before each dosing, urine pregnancy tests will be performed using a commercial kit at the site.
g Only SAEs.
h Trial subjects will be contacted by phone for calls at 5±2 h after each IMP dose.
i Local reactogenicity assessed by the investigator pre-dose and up to 1 h after dosing. Further details are provided in Section 8.3.6.1.
j Trial subjects will be contacted by phone at 14 (+1) d after each IMP dose and will specifically be asked about potential delayed local injection site reactions starting after 7 d and through 14 d post-IMP administration. Further details are provided in Section 8.3.10.
k ECG will be performed pre-dose on the dosing days. Further details are provided in Section 8.3.3.
l Only SAEs and MAAEs.
m In the setting of a temporary trial pause, the visit window for subjects for V 13 (Dose 3) in Cohorts 1-9 can be extended for an additional 23 d (dosing window -7 d/+30 d).

Notes:

All subjects that are early terminated from treatment should undergo regularly scheduled activities through 28 d post last received IMP dose, per SoA (Section 1.3). After this point, subjects that are discontinued from trial treatment after Dose 1 should then receive a safety phone call at 6 months (±14 d) and 12 months (±14 d) post last received IMP dose. Subjects that are discontinued from trial treatment after Dose 2 should perform all visits and assessments per protocol, except for IMP-related procedures. The site investigator will receive a guidance document detailing the applicable assessments of treatment-discontinued subjects and can consult the trial Medical Monitor in case of uncertainty. In cases of pregnancy and other instances where further blood draws may be medically contraindicated in the opinion of the site investigator, no blood draws will be performed during the follow-up.

The total blood volume drawn over any 8-week period in any cohort will always be less than 550 mL. Additional blood samples may be taken, e.g., for safety assessments after AEs or SAEs. The total volume of blood drawn from each subject over 78 weeks will be up to ~1,140 mL.

As long as the volume of blood drawn over any 8-week period is not increased, and as long as the maximum blood volume drawn per day does not exceed the cumulative blood volume specified for that visit in the SoA, the volume of individual blood draws may be adapted if required, e.g., due to unforeseen shortage of specific tubes.

Abbreviations: AE = adverse event; CMI = cell mediated immune response; D = dose; d = day(s); ECG = electrocardiogram; ET = early termination; h = hour(s); β-HCG = beta human chorionic gonadotropin; HLA = human leukocyte antigen; IMP = investigation medicinal product; MAAE = medically attended adverse event; NA = not applicable; SAE = serious adverse event; Scr = screening; SoA = schedule of activities; V = visit; VOCBP = volunteers of childbearing potential.

Table 3: Schedule of activities for Cohort 10 (Part A)

Activity	**Vidit (V) 0**	**V 1**	**V 2 (Call)**	**V 3 (Call)**	**V 4**	**V 5 (Call)**	**V 6**	**V 7 (Call)**	**V 8 (Call)**	**V 9**	**V 10 (Call)**	**V 11**	**V 12 (Call)**	**V 13 (Call)**	**V 14**	**V 15 (Call)**	**V 16**	**V 17**	**V 18**	**V 19**
Visit description	**Scr**	**Dose (D) 1 Day 1**	**24 h post-D 1**	**48 h post-D 1**	**7 d post-D 1**	**14 d post-D 1**	**D 2 (28 d post-D 1)**	**24 h post-D 2**	**48 h post-D 2**	**7 d post-D 2**	**14 d post-D 2**	**D 3 (28 d post-D 2)**	**24 h post-D 3**	**48 h post-D 3**	**7 d post-D 3**	**14 d post-D 3**	**28 d post-D 3**	**84 d post-D 3**	**168 d post-D 3**	**365 d post-D 3 or ET**
Visit window	**up to 42 d before V 1**	**NA**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±14 d**	**±14 d**	**±14 d**
Informed consent	X																			
Inclusion / exclusion criteria	X	X review																		
Medical history	X	X update																		
Physical exam.	X	X [a]			X [a]		X [a]			X [a]		X [a]			X [a]					
Height & body weight	X																			
Vital signs	X	X [b]			X		X [b]			X		X [b]			X					
12-lead ECG [k]	X	X			X		X			X		X			X					
Blood draw for viral screen [c]	13 mL																			
Blood for clinical lab. [d]	6 mL	4.5 mL [e]			4.5 mL		4.5 mL [e]			4.5 mL		4.5 mL [e]			4.5 mL					
Pregnancy test for VOCBP [f]	X	X					X					X								
Randomization to IMP or placebo		X																		

Activity	**Vidit (V) 0**	**V 1**	**V 2 (Call)**	**V 3 (Call)**	**V 4**	**V 5 (Call)**	**V 6**	**V 7 (Call)**	**V 8 (Call)**	**V 9**	**V 10 (Call)**	**V 11**	**V 12 (Call)**	**V 13 (Call)**	**V 14**	**V 15 (Call)**	**V 16**	**V 17**	**V 18**	**V 19**
Visit description	**Scr**	**Dose (D) 1 Day 1**	**24 h post-D 1**	**48 h post-D 1**	**7 d post-D 1**	**14 d post-D 1**	**D 2 (28 d post-D 1)**	**24 h post-D 2**	**48 h post-D 2**	**7 d post-D 2**	**14 d post-D 2**	**D 3 (28 d post-D 2)**	**24 h post-D 3**	**48 h post-D 3**	**7 d post-D 3**	**14 d post-D 3**	**28 d post-D 3**	**84 d post-D 3**	**168 d post-D 3**	**365 d post-D 3 or ET**
Visit window	**up to 42 d before V 1**	**NA**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±14 d**	**±14 d**	**±14 d**
IMP or placebo injection		X					X					X								
Record pregnancies		X	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Counsel / remind subjects to use contraception	X	X			X		X			X		X			X		X	X		
Prior medications and vaccinations	X	X update																		
Record concomitant medications and vaccinations since last visit		X			X		X			X		X			X		X	X	X	X
Blood for humoral response assessments		30 mL [e]					30 mL [e]			30 mL		30 mL [e]			30 mL		30 mL	30 mL	30 mL	30 mL
Blood for CMI assessment		100 mL [e]					60 mL [e]			100 mL		60 mL [e]			100 mL		60 mL	70 mL	70 mL	70 mL

Activity	**Vidit (V) 0**	**V 1**	**V 2 (Call)**	**V 3 (Call)**	**V 4**	**V 5 (Call)**	**V 6**	**V 7 (Call)**	**V 8 (Call)**	**V 9**	**V 10 (Call)**	**V 11**	**V 12 (Call)**	**V 13 (Call)**	**V 14**	**V 15 (Call)**	**V 16**	**V 17**	**V 18**	**V 19**
Visit description	**Scr**	**Dose (D) 1 Day 1**	**24 h post-D 1**	**48 h post-D 1**	**7 d post-D 1**	**14 d post-D 1**	**D 2 (28 d post-D 1)**	**24 h post-D 2**	**48 h post-D 2**	**7 d post-D 2**	**14 d post-D 2**	**D 3 (28 d post-D 2)**	**24 h post-D 3**	**48 h post-D 3**	**7 d post-D 3**	**14 d post-D 3**	**28 d post-D 3**	**84 d post-D 3**	**168 d post-D 3**	**365 d post-D 3 or ET**
Visit window	**up to 42 d before V 1**	**NA**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±14 d**	**±14 d**	**±14 d**
Blood for HLA typing		4 mL [e]																		
Subject hotline availability	Start	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Issue, train and collect subject e-diaries		Issue, train					Re-train					Re-train			Collect					
Subjects report reactogenicity daily for 7 d using an e-diary		Start	=>	=>	End		Start	=>	=>	End		Start	=>	=>	End					
Investigators review e-diary data daily		Start	=>	=>	End		Start	=>	=>	End		Start	=>	=>	End					
Record AEs, MAAEs, and SAEs	X [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X [l]	X [l]	X [l]
Subject phone call		X [h]	X	X		X [j]	X [h]	X	X		X [j]	X [h]	X	X		X [j]				
Investigator local reaction assessment		X [i]					X [i]					X [i]								

Activity	Vidit (V) 0	V 1	V 2 (Call)	V 3 (Call)	V 4	V 5 (Call)	V 6	V 7 (Call)	V 8 (Call)	V 9	V 10 (Call)	V 11	V 12 (Call)	V 13 (Call)	V 14	V 15 (Call)	V 16	V 17	V 18	V 19
Visit description	**Scr**	**Dose (D) 1 Day 1**	**24 h post-D 1**	**48 h post-D 1**	**7 d post-D 1**	**14 d post-D 1**	**D 2 (28 d post-D 1)**	**24 h post-D 2**	**48 h post-D 2**	**7 d post-D 2**	**14 d post-D 2**	**D 3 (28 d post-D 2)**	**24 h post-D 3**	**48 h post-D 3**	**7 d post-D 3**	**14 d post-D 3**	**28 d post-D 3**	**84 d post-D 3**	**168 d post-D 3**	**365 d post-D 3 or ET**
Visit window	**up to 42 d before V 1**	**NA**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±4 h**	**±6 h**	**+1 d**	**+3 d**	**+4 d**	**±14 d**	**±14 d**	**±14 d**
Blood volume at visit (mL)	**19**	**138.5**			**4.5**		**94.5**			**134.5**		**94.5**			**134.5**		**90**	**100**	**100**	**100**

a Brief (symptom-targeted) physical examination, if clinically indicated. If the subject has no symptoms and a physical examination is not clinically indicated, it is not required. Further details are provided in Section 8.3.1.

b On dosing days to be taken within 1 h pre-dose and at 1 h (±15 minutes) post-dose. Further details are provided in Section 8.3.2.

c Viral screening details are provided in Section 8.3.5.

d Clinical laboratory tests details are provided in Section 8.3.4.

e On days with dosing, blood draws should be done pre-dose.

f VOCBP: The serum β-HCG pregnancy test at Visit 0 is performed using the sample collected for clinical laboratory tests. Before each dosing, urine pregnancy tests will be performed using a commercial kit at the site.

g Only SAEs.

h Trial subjects will be contacted by phone for calls at 5±2 h after each IMP dose.

i Local reactogenicity assessed by the investigator pre-dose and up to 1 h after dosing. Further details are provided in Section 8.3.6.1.

j Trial subjects will be contacted by phone at 14 (+1) d after each IMP dose and will specifically be asked about potential delayed local injection site reactions starting after 7 d and through 14 d post-IMP administration. Further details are provided in Section 8.3.10.

k ECG will be performed pre-dose on the dosing days. Further details are provided in Section 8.3.3.

l Only SAEs and MAAEs.

Notes:

All subjects that are early terminated from treatment should undergo regularly scheduled activities through 28 d post last received IMP dose, per SoA (Section 1.3). After this point, subjects that are discontinued from trial treatment after Dose 1 should then receive a safety phone call at 6 months (±14 d) and 12 months (±14 d) post last received IMP dose. Subjects that are discontinued from trial treatment after Dose 2 should perform all visits and assessments per protocol, except for IMP-related procedures. The site investigator will receive a guidance document detailing the applicable assessments of treatment-discontinued subjects and can consult the trial Medical Monitor in case of uncertainty. In cases of pregnancy and other instances where further blood draws may be medically contraindicated in the opinion of the site investigator, no blood draws will be performed during the follow-up.

The total blood volume drawn over any 8-week period in any cohort will always be less than 550 mL. Additional blood samples may be taken, e.g., for safety assessments after AEs or SAEs. The total volume of blood drawn from each subject over 66 weeks will be up to ~1,010 mL.

As long as the volume of blood drawn over any 8-week period is not increased, and as long as the maximum blood volume drawn per day does not exceed the cumulative blood volume specified for that visit in the SoA, the volume of individual blood draws may be adapted if required, e.g., due to unforeseen shortage of specific tubes.

Abbreviations: AE = adverse event; CMI = cell mediated immune response; D = dose; d = day(s); ECG = electrocardiogram; ET = early termination; h = hour(s); β-HCG = beta human chorionic gonadotropin; HLA = human leukocyte antigen; IMP = investigation medicinal product; MAAE = medically attended adverse event; NA = not applicable; SAE = serious adverse event; Scr = screening; SoA = schedule of activities; V = visit; VOCBP = volunteers of childbearing potential.

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ABBREVIATIONS/TERMS

Abbreviation/Term	Explanation
~	Approximately
AE	Adverse event
CHMI	Controlled human malaria infection
COVID-19	Coronavirus Disease 2019
CRF	Case report form
CRO	Contract research organization
CSP	Circumsporozoite protein
d	Day(s)
ECG	Electrocardiogram
EDC	Electronic data capture (system)
Enrolled subjects	Subjects who signed an informed consent form, i.e., who gave informed consent
EU	European Union
h	Hour(s)
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HLA	Human leukocyte antigen
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Council for Harmonisation (of Technical Requirements for Pharmaceuticals for Human Use)
IDMC	Independent Data Monitoring Committee
IM	Intramuscular or intramuscularly
IMP	Investigational medicinal product
IRB/IEC	Institutional Review Boards/Independent Ethics Committees
IRC	Internal Review Committee
*Pf*CSP	*Plasmodium falciparum* circumsporozoite protein
QTcF	The corrected QT interval by Fridericia
SAE	Serious adverse event
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
SoA	Schedule of activities
SUSAR	Suspected unexpected serious adverse reaction
VE	Vaccine efficacy
VOCBP	"Volunteers" of childbearing potential is used instead of "women" of childbearing potential to encompass all volunteers born female
US FDA	United States Food and Drug Administration
WHO	World Health Organization

2 INTRODUCTION

2.1 Trial rationale

The WHO estimated that there were ~249 million cases of malaria and 608,000 malaria-associated deaths in 2022, the majority of which were caused by the parasite *P. falciparum* (WHO Malaria Report 2023). The WHO is aiming to reduce the incidence and mortality due to malaria by 90% by 2030. Despite programmatic efforts, recent gains to decrease malaria morbidity and mortality have been partially reversed, underscoring the need for effective malaria vaccines and other new tools to prevent malaria and achieve the WHO goals.

The world's first WHO-recommended malaria vaccine (RTS,S/AS01; Mosquirix™) received favorable opinion from the EMA in 2015. It has a relatively low vaccine efficacy (VE) in preventing clinical malaria cases (it prevented ~36% of cases of clinical malaria over a median follow-up of 48 months in children aged 5 to 17 months) and its efficacy also wanes over time (RTS,S Clinical Trials Partnership 2015). The WHO has encouraged the development of new and improved next-generation malaria vaccines with higher efficacy and improved durability.

In 2023 the WHO recommended R21/Matrix-M for the prevention of malaria in children living in endemic regions. R21/Matrix-M is similar to Mosquirix since it is also composed of the same region of *Pf*CSP fused to the Hepatitis B surface antigen (HBsAg) (SAGE Meeting Report 2023). Based on a Phase III study of R21/Matrix-M, VE against clinical malaria was 75% over 12 months at seasonal sites and 68% at standard sites. A booster dose at seasonal sites given at 12 months resulted in 74% VE at 18 months (Datoo et al. 2024). Longer-term follow-up data for R21/Matrix-M is not yet available.

BioNTech aims to develop a highly efficacious *P. falciparum* blood stage infection prevention vaccine that at an individual level prevents disease and death. When this vaccine is applied to a large enough proportion of the target population constituting the infectious reservoir, it may prevent onward transmission, and thus enable malaria elimination. The vaccine under development is intended to induce poly-specific humoral and cellular immune responses against multiple *P. falciparum* antigens expressed in different stages of its life cycle rather than relying on targeting sporozoites alone, as done by Mosquirix™.

For the development of this malaria vaccine BioNTech will use the RNA technology platform used for the SARS-CoV-2 vaccine BNT162b2 "COMIRNATY" which has been licensed for emergency use or been given conditional or full marketing authorizations in more than 100 countries globally. The LNP carrier system, the chemistry (nucleoside-modified) and the sequence design of the RNA molecule's untranslated backbone elements (cap, untranslated regions, polyA tail) will remain unchanged. Only the encoded open reading frames will change and will represent *P. falciparum* derived antigens. Also, the same well characterized manufacturing process, controls and possibly also manufacturing facilities, used for BNT162b2 will be utilized, as well as the same route of administration.

BioNTech has started clinical development of a malaria vaccine with the trial BNT165-01 that assesses safety, reactogenicity, and immunogenicity of BNT165b1, an RNA encoding part of the *Pf*CSP (NCT05581641).

The malaria vaccine candidate BNT165e tested in this clinical trial is a combination of three distinct RNAs, BNT165c and BNT165d (composed of BNT165d1 and BNT165d2), encoding *P. falciparum* antigens each encapsulated in the LNP. BNT165d1 and BNT165d2 both encode conserved, immunogenic segments of liver-stage expressed proteins. BNT165c encodes the full *Pf*CSP.

We will start the clinical development of BNT165e with this trial BNT165-02 with Part A evaluating the safety, tolerability and immunogenicity of different dose combinations of BNT165c and BNT165d. Some cohorts will assess BNT165c and BNT165d on their own to evaluate their contribution to the safety and immunogenicity profile of BNT165e. Neither BNT165c nor BNT165d are intended to be developed as individual vaccine candidates and will be used only in the context of BNT165e.

CCI [redacted]

For the rationale for a scientific rationale for the trial design, see Section 4.2.

For the rationale for the trial treatment, see Section 6.3.

2.2 Background

2.2.1 The medical need

Malaria is a serious and sometimes fatal disease caused by *Plasmodium spp.* parasites, which are transmitted to humans by *Anopheles* mosquitoes. Of all human pathogen *Plasmodium* spp., *P. falciparum* is most likely to lead to severe disease and death. Eighty-five countries were considered malaria-endemic in 2022, and most of these countries are located in Sub-Saharan Africa (WHO Malaria Report 2023). The WHO African region accounted for 93.6% of all malaria cases in 2022 and 95.4% of deaths due to malaria worldwide. Seventy-nine percent of all malaria-related deaths in this region were in children aged 5 years and younger (WHO Malaria Report 2023). Four African countries account for almost half of all global malaria cases: Nigeria, the Democratic Republic of the Congo, the United Republic of Tanzania, and Niger. Thirty-eight percent of all deaths due to malaria worldwide in children aged 5 years and under occurred in Nigeria in 2022.

Furthermore, an estimated 12.7 million pregnancies in the WHO African Region were exposed to malaria infection, putting them at increased risk for adverse pregnancy outcomes. Malaria exposure in pregnancy was estimated to have led to 392,806 neonates with low birthweight in 2022 (WHO Malaria Report 2023).

Other world regions have a minor contribution to global malaria cases, and each contribute less than 5 million cases per year. In decreasing order of incidence, malaria-endemic regions are: WHO South-East Asia region (mainly India), WHO Eastern Mediterranean region, WHO Western Pacific Region and WHO Region of the Americas. The WHO European region has been malaria-free since 2015. In 2015, the WHO established the goal to reduce global malaria incidence and death rates by 90% by 2030 to accelerate malaria eradication. There have been successes in reducing the burden of malaria since the early 2000s, with 24 countries declared malaria-free since and no re-introduction of malaria in any of them.

Efforts to reduce malaria incidence are multi-pronged and include distribution of insecticide-treated bed nets, indoor residual spraying, treatment with perennial or seasonal

malaria prophylaxis, intermittent preventive treatment in pregnancy, and expansion of diagnosis and treatment. With the WHO recommendation to use RTS,S/AS01 in children aged 5 to 17 months in high to moderate malaria-transmission settings, vaccination is an additional tool for reduction in malaria incidence.

Despite considerable progress in reduction of malaria incidence and mortality rates since the 2000s, progress has stalled in recent years, and, due to disruptions in services due to the COVID-19 pandemic, even reversed to some extent. If these trends continue, there is a high risk that the WHO eradication goals for 2030 will not be achieved, especially in the WHO African region countries.

Additionally, there is a risk of development of biological resistance against insecticides and antimalarial treatments. Highly effective vaccines preventing malaria caused by *P. falciparum* are urgently needed to achieve the goal of malaria eradication and to reduce disease and the socioeconomic burden of malaria in highly endemic areas.

In non-endemic countries, the majority of malaria cases are imported from malaria-endemic areas by tourists, migrant workers or immigrants returning from visiting friends and relatives. Travelers can protect themselves from malaria by taking chemoprophylaxis (e.g., atovaquone/proguanil, doxycycline, mefloquine), however, this may be impractical for prolonged stays. Ultimately over 90% of imported malaria cases occur in individuals who did not take or incorrectly took chemoprophylaxis. A highly efficacious long-lasting malaria vaccine could also help prevent imported *P. falciparum* malaria cases, which are increasing annually in the US (Malaria surveillance 2018).

The world's first WHO-recommended malaria vaccine is an adjuvanted protein subunit vaccine that consists of the major repeat region and the C-terminus of the *Pf*CSP fused to the HBsAg. The vaccine is a mix of this *Pf*CSP-HBsAg compound with HBsAg that forms virus-like particles (RTS,S/AS01; Mosquirix™). Phase III studies of RTS,S delivered as a 3-dose series with a booster after 1 year showed moderate VE in children aged 5 to 17 months preventing 36% of clinical malaria cases over the full study period with a median follow-up of 4 years, with a range of 20% in high to 66% in low transmission settings. Furthermore, published literature suggests that protection wanes over time and there are reports of negative efficacy after 5 years in children with high malaria exposure (Olotu et al. 2016).

R21/Matrix-M is another malaria vaccine that was recommended by the WHO in 2023 for use in children. R21/Matrix-M is based on the same immunological target as RTS,S/AS01 and has comparable clinical activity. The WHO has encouraged the development of new and improved next-generation malaria vaccines with higher efficacy and improved durability compared to first-generation vaccines to achieve their stated goals (R21/Matrix-M, WHO 2023).

2.2.2 Introduction to the IMP

BioNTech plans to develop a vaccine for active immunization against *P. falciparum* malaria that can be rapidly manufactured at scale.

As demonstrated by the SARS-CoV-2 vaccine BNT162b2 (COMIRNATY), the development of vaccines with RNA-LNPs encoding antigens from a target pathogen that are translated to protein by the vaccinated individual can induce a protective immune response and provides significant benefits, including:

- **Safety:** Based on the current understanding of the safety experience with BNT162b2, mRNA-based vaccines are expected to have a tolerable safety profile both in the general population and in special populations such as immunocompromised individuals.
- **Multi-antigen vaccines:** P. falciparum displays a multitude of surface proteins over its complex life cycle. The BioNTech RNA technology platform allows the expression of multiple antigens and hence is well suited to develop a multivalent vaccine. This versatility has prompted development of an RNA-based malaria vaccine that will target both the sporozoite and the liver stage.

BioNTech SE has started clinical development of malaria vaccine with the BNT165-01 trial, which assesses the safety, reactogenicity, and immunogenicity of BNT165b1, an RNA encoding a part of *Pf*CSP (NCT05581641). This trial BNT165-02 will open the clinical development of a different candidate BNT165e.

BNT165e consists of three distinct RNAs (BNT165c, BNT165d1, and BNT165d2) encoding different *P. falciparum* antigens encapsulated in the LNPs. BNT165c encodes *Pf*CSP. BNT165d1 and BNT165d2 both encode conserved, immunogenic segments of liver-stage expressed proteins. Throughout the development, BNT165d denotes a combination at 1:1 ratio of both BNT165d1 and BNT165d2. The trial BNT165-02 will start with Part A to evaluate the safety, tolerability, and immunogenicity of different dose combinations of BNT165c and BNT165d. CCI [redacted] Some cohorts assess BNT165c and BNT165d on their own to assess their contribution to the safety and immunogenicity profile of BNT165e, but neither BNT165c nor BNT165d are intended to be developed as individual vaccine candidates and will be used only in the context of BNT165e.

For more details about the BNT165e vaccine, including the targeted antigens, see the BNT165e IB. The final BioNTech vaccine for Phase II/III clinical development will be selected based on aggregate data from pre-clinical studies and clinical testing.

2.3 Benefit/risk assessment

More detailed information about the known and expected benefits and risks, and reasonably expected AEs for this trial are given in the BNT165e IB.

2.3.1 Benefit assessment

Trial subjects will not benefit directly from participation in this trial, but by participating, they will be contributing to general knowledge of RNA-based malaria vaccines.

The potential benefits of receiving BNT165e have not been demonstrated, but by undergoing laboratory tests and physical examinations in this trial, previously undiagnosed health problems may be uncovered.

2.3.2 *Risk assessment and mitigation strategy*

Potential risk source	Summary of data/Rationale for risk	Mitigation strategy
General risks linked with vaccinations		
General risks	Rarely, a vaccine may cause allergic reactions such as rash or difficulty breathing. Allergic reactions may be life-threatening.	• Subjects with known allergies against the vaccine or COMIRNATY or its excipients will be excluded from trial participation. • All subjects will be observed at the site for at least 1 h after each IMP injection. • Equipment to treat anaphylaxis will be readily available.
General risks	Side effects seen from giving a vaccine by IM injection into the muscle include local reactions at the injection site such as redness, itchiness, tenderness, pain, and swelling.	• Injection sites will be monitored for at least 1 h after each IMP injection. • Additionally, subjects will report local reactogenicity for 7 d using e-diaries and be contacted 14 d post-dose to check for any late reactogenicity events.
General risks	All vaccines can cause fever, chills, rash, aches and pains in the muscles or joints, nausea and loss of appetite, headache, dizziness, and feeling tired. Most people are still able to do their planned activities after getting a vaccine. Rarely, people experience side effects that limit their normal activities or make them go to their doctor.	• In general, these risks can be managed using routine symptom-driven standard of care. Treatment of these events is dependent on the discretion of the investigators. For suggestions of management, please refer to Section 6.10.2. • All subjects will be observed at the site for at least 1 h after each IMP injection. • Additionally, subjects will report systemic reactogenicity for 7 d using e-diaries.
Trial treatment: IMP		
IMP related risks	Risks related to the administration of an IMP. The BNT165d1 and BNT165d2 candidates contain MITD sequences, there is limited clinical experience with use of MITD in vaccines. As the MITD drives improved presentation of antigens in the MHC Class I compartment, it may lead to a risk of immune-mediated diseases / conditions / autoimmune conditions or worsening of autoimmune conditions.	• The medical monitor and the IRC will regularly review and evaluate the safety data including any emerging AEs, MAAEs or SAEs. Pausing rules include any AE that may be an immune-mediated disease, including but not limited to myocarditis, pericarditis or myopericarditis or other significant immune-mediated condition that may result in end-organ damage, be life-threatening, or otherwise result in significant downstream health consequences that is assessed as related by the investigator, or for which there is no alternative, plausible, attributable cause (see Section 7.1.1).
Pregnancy-related risks	Currently no data on human teratogenicity/fetotoxicity is available.	• The investigator or designee will inform the trial subject of the need to use highly effective contraception consistently and correctly.

Potential risk source	Summary of data/Rationale for risk	Mitigation strategy
		Restrictions apply for the donation and/or cryopreservation of germ cells. • Pregnancy tests are being conducted throughout the trial at screening and at every IMP timepoint as a risk mitigation strategy.
Trial procedures		
Venipuncture and blood draws	There is the risk of bleeding, bruising, hematoma formation, and infection at the venipuncture site. Subjects may experience pain or feel faint.	• Venipuncture will only be performed by qualified trial site personnel. • The volume of blood drawn at any visit is kept to the amounts required to support the objectives of this trial (Section 1.3). The volume of blood drawn per day and overall is restricted.

Abbreviations: AE = adverse event; d = day(s); ECG = electrocardiogram; h = hour(s); IM = intramuscular; IMP = investigational medicinal product; IRC = Internal Review Committee; MAAE = medically attended adverse event; MHC = major histocompatibility complex; MITD = MHC Class I targeting domain; SAE = serious adverse event.

2.3.3 Overall benefit/risk conclusion

The potential risk to trial subjects associated with BNT165e is considered as low. The trial was designed to minimize risk to the subjects by using sentinel dosing, close monitoring of subjects etc., thus, the potential risks identified in association with the trial procedures and trial treatments are considered justified by the anticipated benefits if a vaccine for the prevention of clinical malaria is successfully developed.

3 OBJECTIVES, ENDPOINTS, AND ESTIMANDS

OBJECTIVES	ENDPOINTS	OUTCOME MEASURES
Primary objective (Part A)		
To describe the safety and tolerability of BNT165e and its components in healthy adults.	• Solicited local reactions (pain, erythema/redness, induration/swelling). • Solicited systemic reactions (vomiting, diarrhea, headache, fatigue, muscle/joint pain, and fever). • AEs • SAEs • MAAEs	For each cohort in subjects receiving at least one dose of trial intervention: • Frequency of solicited local reactions at the injection site recorded up to 7 d after each dose. • Frequency of solicited systemic reactions recorded up to 7 d after each dose. • Frequency of subjects with at least one AE occurring until 28 d after each dose. • Frequency of subjects with at least one MAAE occurring until 24 weeks after last received IMP dose. • Frequency of subjects in each cohort with at least one SAE occurring until 24 weeks after last received IMP dose.
Secondary objective (Part A)		
To evaluate the CSP-specific humoral immune response of BNT165e and its components.	Anti-CSP IgG levels at specified timepoints in the SoA (Section 1.3).	For each cohort: • Descriptive statistics on antibody levels (including geometric means and 95% CI) at assessed timepoints.
Exploratory objective (Part A)		
To evaluate the cell mediated immune response	CD4 and CD8 T-cell responses at baseline and specified timepoints post-immunization (Section 1.3).	• Frequency of antigen-specific T cells at assessed timepoints.

Abbreviations: AE = adverse event; CD = cluster of differentiation; CI = confidence interval; CSP = circumsporozoite protein; d = day(s); IgG = immunoglobulin G; IMP = investigational medicinal product; MAAE = medically attended adverse event; SAE = serious adverse event; SoA = schedule of activities.

4 TRIAL DESIGN

4.1 Overall design

Part A of this trial will evaluate the safety, tolerability, and immunogenicity of a multi-component vaccine BNT165e, consisting of three mRNA components encoding the *Pf*CSP and immunogenic segments of liver-stage expressed proteins.

CCI [redacted]

Part A

In Part A, the combination of the three components will be evaluated in different dose combinations in a 3-dose schedule to select a safe, tolerable, and immunogenic dose combination, and to assess the impact of third dose on immunogenicity. One dose combination will also be tested in a Day 1, 29 and 57 dosing schedule in Cohort 10 (Section 1.3). Part A of the trial will be observer-blind.

Up to 138 healthy subjects are divided into 10 cohorts with different dose combinations and will be randomized 5:1 active:placebo. Number of subjects may be increased up to 177 in the event of subject replacement or re-enrollment.

Table 1 denotes the dose combinations being tested in the different cohorts and the planned number of subjects to be included.

For the planned assessments and visits, see the schedule of activities (SoA) in Section 1.3. This trial will use a staggered dose-escalation schema with sentinel subjects in all cohorts (see Figure 1).

Within all cohorts in Part A, subjects will be randomized in a 5:1 ratio to IMP vs. placebo.

Cohort 1 will be initiated with two subjects as the sentinel group, whereby at least one sentinel subject will receive active IMP. Cohorts 2 to 9 will be initiated with three subjects as the sentinel group, whereby at least two sentinel subjects in each cohort will receive active IMP. The sentinel subjects in one cohort will be dosed at the same clinical trial site at least 1 h apart. The sentinels will complete 48 h of follow-up post-Dose 1. In case the investigator considers it unsafe to continue, an *ad hoc* IRC will be convened to decide on continuation of the cohort. If the investigator considers the vaccine reactogenicity acceptable (only Grade 2 or lower events or after consultation with medical monitor) and no stopping/pausing rules (see Section 7.1.1) are met, the remaining subjects per cohort can be dosed. One of the dose levels tested in Cohorts 1 to 8 will subsequently be tested with a Day 1, Day 29 and Day 57 dosing schedule in Cohort 10, without sentinel dosing.

In Part A, cohorts testing BNT165c alone will be opened first. The first 3 cohorts will be opened sequentially with an IRC meeting before each dose-escalation. Cohort 1 (10 µg BNT165c) will first be opened. If the IRC decides to continue dose-escalation based on all available 7 d post-Dose 1 safety data, then Cohort 2 (≤30 µg BNT165c) will be opened. Similarly, the sentinel subjects will first be dosed, followed by the rest of the cohort after 48 h of follow-up. If the decision is made to open the next cohort, then Cohort 3 (≤70 µg BNT165c) will be opened.

At this point the IRC will convene to assess all available clinical, laboratory and other relevant data.

If no stopping/pausing rules are met and there are no other safety concerns, then Cohort 9 (≤100 µg BNT165c) will be opened. Alternatively, if the IRC decides not to open Cohort 9, then Cohort 4 will be opened next instead. If Cohort 9 is opened, then it will be filled first. Thereafter, Cohort 4 (30 µg BNT165d) will be opened. After Cohort 4 is filled, Cohort 5 (≤30 µg BNT165c and 10 µg BNT165d) will be opened. Once all subjects in Cohorts 9 (if opened), 4 and 5 have completed 7 d follow-up post-Dose 1, all available clinical, laboratory and other relevant data will be reviewed by the IRC. If the IRC considers the safety profile of the IMP to be acceptable and no stopping/pausing rules are met, dosing will be opened for Cohort 6 (≤10 µg BNT165c and ≤30 µg BNT165d). After this cohort is filled, Cohort 7 (≤30 µg BNT165c and ≤30 µg BNT165d) will be opened. After Cohort 7 is filled, Cohort 8 (≤70 µg BNT165c and ≤30 µg BNT165d) will be opened.

Based on all the safety data available until at least 28 d after Cohorts 1 to 9 have received their first dose, the IRC will decide on a dose level for Cohort 10 from one of the dose levels used in Cohorts 1 to 8. Cohort 10 will subsequently be dosed with IMP on Day 1, Day 29 and Day 57.

Provided no cohort trial treatment pausing rules are met, the reactogenicity profile is acceptable and the IRC does not identify safety findings of clinical concern, subjects who received Dose 1 in each cohort will proceed to receive Dose 2 and Dose 3. If any of the pausing criteria are met, no additional subjects will be assigned to the trial and administration of IMP for all cohorts, including administration of Dose 2 and Dose 3 for all cohorts, will be paused until the IRC reviews the event(s).

The IRC may recommend that the planned escalation dose combination be reduced for a given cohort. Other unplanned dose modifications, pausing (temporary halting) of trial treatment, or even discontinuation of trial treatment may be required. See Section 7 for guidance on criteria for such cases.

In addition to the IRC, the trial medical monitor will review the clinical and safety data on an ongoing basis, interact with trial sites as needed, and assess whether any pausing rules are met. The IDMC will review summary safety data of all subjects in the clinical trial on a quarterly basis according to the IDMC charter.

Suspected unexpected serious adverse reactions (SUSARs) and possible cases of myocarditis, pericarditis or myopericarditis will trigger an *ad hoc* review by the IDMC. Possible cases of myocarditis, pericarditis, or myopericarditis will be independently assessed by the IDMC using the Brighton Collaboration Case Definition.

For a graphical depiction of the trial, see Figure 2.

The duration of the trial periods is given in Section 4.1.1.

The planned number of trial subjects is given in Section 4.1.2.

For standard definitions, e.g., for trial completer and end of trial, see Section 4.3.

4.1.1 Duration of the trial periods

The planned trial duration for each subject in Cohort 1 to 9 is up to 6 weeks screening, 26 weeks vaccination phase, and 52 weeks follow-up phase, amounting to a maximum of 84 weeks.

The planned trial duration for each subject in Cohort 10 is up to 6 weeks screening, 8 weeks vaccination phase, and 52 weeks follow-up phase, amounting to a maximum of 66 weeks.

Trial duration may exceed the listed maximum time in the event of a trial pause.

4.1.2 Planned number of trial subjects

In Part A, up to 138 subjects will be enrolled, in cohorts as defined in Table 1. Number of subjects may be increased up to 177 in the event of subject replacement or re-enrollment. The sample size for each cohort is mainly driven by a dose-escalation trial in a limited number of subjects designed for early detection of potential safety and reactogenicity events, and is considered adequate to support the trial objectives, while minimizing the number of trial subjects exposed to a new IMP.

If a subject is discontinued either from further vaccination or discontinued from the trial, the sponsor can decide to replace this subject with a new subject. Subjects will be randomized to the vacancy in the open cohort until that cohort has filled.

4.2 Rationale for the trial design

This trial will start the clinical development of BNT165e.

Part A is designed to evaluate the safety, tolerability, and immunogenicity of different dose combinations of BNT165c and BNT165d (BNT165d1 and BNT165d2 will always be used in a dose ratio of 1:1). Since BNT165e and its components will be evaluated for the first time, this trial uses a staggered, sequential approach for subject dosing. Dosing will proceed with an initial sentinel group for each new dose level cohort, pause/stopping rules will be followed, and there will be ongoing monitoring by a trial IRC that may pause, permanently discontinue administration of trial treatment, or even permanently terminate the trial at any time.

The starting doses in the first four cohorts are lower than or equal to the dose approved for BNT162b1 (30 µg) and are lower than or equal to those used in the BNT165-01 malaria vaccine trial (NCT05581641). Subsequent dose cohorts will have incrementally higher doses. Part A will test a three-dose regimen to assess the impact of a third dose on immunogenicity markers. The subjects will be followed up for 1 year after Dose 3 to better understand the kinetics and durability of the immune response.

Some cohorts will assess BNT165c and BNT165d on their own to assess their contribution to the safety and immunogenicity profile of BNT165e, and to detect any potential interaction on the outcome measures by the components. Neither BNT165c nor BNT165d are intended to be developed as individual vaccine candidates but will be used only in the context of BNT165e.

Part A uses an observer-blinded, placebo-controlled design, as this enables a bias-free estimation of reactogenicity, which is a main driver of the safety profile and may influence dose selection for Part B and further development of BNT165e.

CCI

4.2.1 Rationale for the level of contraception

Currently there is no data on human teratogenicity/fetotoxicity. Therefore, all trial subjects biologically capable of having or fathering children must agree to use a highly effective method of contraception consistently and correctly as specified in the SoA (Section 1.3). In addition, pregnancy testing will be performed as specified in the SoA (Section 1.3).

At time points indicated in the SoA (Section 1.3), the investigator or designee will inform the trial subject of the need to use the contraception consistently and correctly, and will counsel the subject not to donate or cryopreserve germ cells. In addition, the investigator or designee will instruct the trial subject to call immediately if the selected contraception method is discontinued or if pregnancy is known or suspected in the trial subject or partner. The investigator or designee will document this conversation at the time points indicated in the SoA (Section 1.3).

The investigator or designee, in consultation with the trial subject, will confirm that the trial subject has selected an appropriate method of contraception for the individual trial subject or his or her partner(s) from the permitted list of contraception methods (Section 10.5.2), and will confirm that the trial subject has been instructed in its consistent and correct use.

The volunteers of childbearing potential (VOCBP) will only be administered trial treatment after negative pregnancy test outcomes at the time points indicated in the SoA (Section 1.3).

For definitions of VOCBP, postmenopausal female and fertile men, as well as guidance on how to collect pregnancy information, see Section 10.5.3.

4.3 Definition of start of trial and end of trial

The “start of trial” date is when the first potential trial subject has given informed consent, see Section 10.1.3.

The “end of the trial” date is when the last visit (or procedure) of the last trial subject in the trial globally.

For guidance on trial discontinuation, see Section 10.1.9.

5 TRIAL POPULATION

The following eligibility criteria are designed to select trial subjects for whom participation in the trial is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether a particular trial subject is suitable for enrollment.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

Investigators should always use good clinical judgment in considering a volunteer’s overall fitness for trial participation. Some volunteers may not be appropriate for enrollment even if they meet all inclusion/exclusion criteria. Medical, psychiatric, occupational, or other conditions may make evaluation of safety and/or immunogenicity difficult, and some volunteers may be poor candidates for retention.

Determination of eligibility, considering all inclusion and exclusion criteria, must be made within 42 d before enrollment unless otherwise noted in the following sections.

There must be documentation supporting compliance with the following listed inclusion/exclusion criteria, e.g., interview feedback, outcomes of trial procedures, medical records, etc., that confirm volunteers are eligible for inclusion in this trial.

5.1 Inclusion criteria

Volunteers are eligible to be included in the trial if all of the following criteria apply for the respective part of the trial:

Inclusion criteria (Part A)

1 Have given informed consent by signing and dating the informed consent form (ICF) before initiation of any trial-specific procedures.

2 Are willing and able to comply with scheduled visits, treatment schedule, laboratory tests, lifestyle restrictions and other requirements of the trial. This includes that they are able to understand and follow trial-related instructions.

3 Are aged 18 to 55 years, have a body mass index over 18.5 kg/m^2 and under 35 kg/m^2 and weigh at least 45 kg at Visit 0.

4 Are healthy, in the clinical judgment of the investigator based on volunteer-reported medical history data, and physical examination, 12-lead electrocardiogram (ECG), vital signs, and clinical laboratory test outcomes at Visit 0.

Note: Healthy volunteers with pre-existing stable disease (e.g., obesity, hypertension), defined as disease not requiring significant change in therapy or hospitalization for worsening disease during the 90 d before Visit 0, can be included.

5 Agree not to enroll in another trial with an IMP starting from Visit 0 and until 12 weeks after receiving Dose 3.

6 Have not traveled and agree not to travel to a malaria-endemic region, as defined per Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/malaria/travelers/country_table/a.html) starting 6 months before Visit 0 and continuously until 28 d after receiving Dose 3.

Virology

7 Negative human immunodeficiency virus (HIV) -1 and -2 blood test result at Visit 0.

8 Negative Hepatitis B surface antigen (HBsAg) test result at Visit 0 and negative anti-Hepatitis C virus (anti-HCV) antibodies, or negative HCV PCR test result if the anti-HCV is positive at Visit 0.

Reproductive status and contraception

9 VOCBP that have a negative serum β-human chorionic gonadotropin (HCG) pregnancy test result at Visit 0 and negative urine pregnancy test results before each IMP administration. Volunteers born female who are postmenopausal or permanently sterilized will not be considered VOCBP.

10 VOCBP who agree to practice a highly effective form of contraception (see Section 10.5.2) starting at Visit 0 and continuously until 90 d after receiving Dose 3.

11 VOCBP who agree not to donate or cryopreserve eggs (ova, oocytes) for the purposes of assisted reproduction during trial, starting at Visit 0 and continuously until 90 d after receiving Dose 3.

12 Men who have not had a vasectomy and are sexually active with partners of childbearing potential and who agree to use condoms and to practice a highly effective form of contraception with their sexual partners of childbearing potential (see Section 10.5.2) during the trial, starting at Visit 0 and continuously until 90 d after receiving Dose 3.

13 Men who are willing to refrain from sperm donation, starting at Visit 0 and continuously until 90 d after receiving Dose 3.

5.2 Exclusion criteria

Volunteers are not eligible to be included in the trial if any of the following criteria apply for the respective part of the trial:

Exclusion criteria (Part A)

1 History of *Plasmodium* parasitemia (any species) based on volunteer-reported medical history.

2 Prior residence for ≥6 months continuously in a malaria-endemic region as defined per CDC (https://www.cdc.gov/malaria/travelers/country_table/a.html) at any point during their lifetime.

3 Breastfeeding or intending to become pregnant or to father children starting with Visit 0 and continuously until 90 d after receiving Dose 3.

4 History of any serious adverse reactions to vaccines or to vaccine components such as lipids, and including history of anaphylaxis and related symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain. (Not excluded from participation: a volunteer who had an anaphylactic adverse reaction to pertussis vaccine as a child).

5 Current or history of the following medical conditions:

a) Uncontrolled or moderate or severe respiratory diseases (e.g., asthma, chronic obstructive pulmonary disease); symptoms of asthma severity as defined in the US National Asthma Education and Prevention Program Expert Panel report, 2020 – e.g., exclude a volunteer who:

- Uses a short-acting rescue inhaler (typically a beta 2 agonist) daily, or
- Uses high dose inhaled corticosteroids (per American Academy of Allergy Asthma & Immunology), or
- In the past year has either of the following:
  - Greater than one exacerbation of symptoms treated with oral/parenteral corticosteroids;
  - Needed hospitalization, or intubation for asthma.

b) History of Diabetes mellitus type 1 or type 2, including cases controlled with diet alone or elevated hemoglobin A1C (HbA1c) ≥6.5% at screening (not excluded: history of isolated gestational diabetes).

c) Hypertension:

- If a person has a history of hypertension, or elevated blood pressure detected during screening, exclude for blood pressure that is not well controlled. Well controlled blood pressure is defined as consistently ≤140 mm Hg systolic and ≤90 mm Hg

diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤150 mm Hg systolic and ≤100 mm Hg diastolic at screening and enrollment.

d) Malignancy within 5 years of screening, excluding localized basal or squamous cell skin cancer;

e) Any current or history of cardiovascular diseases, (e.g., myocarditis, pericarditis, myocardial infarction, congestive heart failure, cardiomyopathy or clinically significant arrhythmias), unless such disease is not considered relevant for participation in this trial in the investigator's judgment;

f) An abnormal screening ECG (i.e., showing the corrected QT interval by Fridericia [QTcF] >450 ms; significant ST-T wave changes suggestive of myocardial ischemia or of an acute or indeterminate-age myocardial infarction; left ventricular hypertrophy; any non-sinus rhythm including isolated premature ventricular contractions; complete right or left bundle branch block [QRS >120 ms]; second-or third-degree atrioventricular [AV] block); or other clinically significant abnormalities on the ECG at the investigator's discretion;

g) Bleeding disorder diagnosed by a doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions);

h) Seizure disorder: History of seizure(s) within past 3 years. Also exclude if volunteer has used medications in order to prevent or treat seizure(s) at any time within the past 3 years.

6 Documented major psychiatric illness, including bipolar disorder, major depressive disorder, schizophrenia, autism, and attention deficit-hyperactivity disorder that at the discretion of the investigator could interfere with participation and follow-up as outlined by the trial.

7 The following diseases associated with immune dysregulation:

- Primary immunodeficiencies.
- History of solid organ or bone marrow transplantation.
- Asplenia: any condition resulting in the absence of a functional spleen.
- Currently existing or history of autoimmune disease including and not limited to thyroid autoimmune disease, multiple sclerosis, psoriasis, etc.

Prior/concomitant therapy

8 Previous vaccination with an approved or investigational malaria vaccine at any time or having taken part in a human malaria challenge study.

9 Receipt of any investigational product within 28 d before Visit 0.

10 Any planned non-trial vaccinations starting at Visit 0 and continuously until 28 d after Dose 3.

Note: Seasonal influenza and COVID-19 vaccines are allowed; however, they should be administered at least 14 d before or 28 d after any IMP administration. Emergency vaccinations, such as tetanus, are allowed to be administered when medically indicated.

11 Received blood/plasma products, monoclonal antibodies or immunoglobulin within 120 d before Visit 1 or planned administration starting at Visit 0 and continuously until the last trial visit (365 d after Dose 3).

12 Received allergy treatment with antigen injections within 28 d before and after each IMP administration.

13 Current or planned treatment with immunosuppressive therapy, including systemic corticosteroids (if systemic corticosteroids are administered for ≥14 d at a dose of ≥20 mg/d of prednisone or equivalent) starting at Visit 0 and continuously until 28 d after Dose 3. Intraarticular, intrabursal, or topical (skin or eyes) corticosteroids are permitted.

Additional exclusions

14 Have a history of alcohol abuse or drug addiction within 1 year before Visit 0 or have a history (within the past 5 years) of substance abuse, which in the opinion of the investigator, could compromise their wellbeing if they participate as subjects in the trial, or that could prevent, limit, or confound the protocol specified assessments.

15 Any existing condition which may affect vaccine injection and/or assessment of local reactions at the injection site, e.g., tattoos, severe scars, etc.

16 Are vulnerable individuals as per International Council for Harmonisation (ICH) E6 definition, i.e., are individuals whose willingness to volunteer in a clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate.

17 Any screening hematology and/or blood chemistry laboratory value (per the US FDA 2007) that meets the definition of a Grade ≥2 abnormality or a Grade 1 abnormality at the investigator's discretion at Visit 0 or a Visit 0 troponin above the reference range. Individuals with abnormal but not clinically significant parameters not included in the toxicity guidance may be considered eligible at discretion of investigator, with the exception of abnormal troponin values.

5.3 Lifestyle considerations

The trial subjects will be required to remain at the site for 1 hour after each IMP administration. When at the trial site, trial subjects will be asked:

- To drink fluids (e.g., water) before each site visit and during the ~2 h after each IMP administration per trial site standard.
- To not smoke or to drink alcohol when on site.

Trial subjects will be asked to avoid strenuous exercise beyond their usual exercise routine for 6 d after each IMP administration after leaving the trial site.

Trial subjects will also be required to practice contraception as outlined in Section 8.4.5 and Section 10.5.2.

5.4 Screen failures

Screen failures are defined as individuals who consent to participate in the trial but who are not subsequently allocated to IMP.

A minimal set of screen failure information is required to ensure transparent reporting of screening failures to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demographic information, date the ICF was signed, the reasons for screen failures, and any serious adverse events (SAEs), if applicable.

If agreed with the trial medical monitor, rescreening is allowed at the discretion of the investigators. Rescreened subjects will need to reconsent and will be assigned a new trial subject number.

5.5 Criteria for temporarily delaying enrollment or randomization

Enrollment or randomization may be paused or delayed if the IRC recommends a pausing of trial treatment according to Section 7.1.1.

6 TRIAL TREATMENTS AND CONCOMITANT THERAPY

Trial treatment is defined as any investigational treatment(s), marketed product(s) intended to be administered to a trial subject according to the trial protocol.

An “IMP” is any medicinal product which is being tested or used as a reference in a clinical trial (the tested product and its reference products, including placebos).

Concomitant therapy is not considered trial treatment.

6.1 Trial treatment(s) administered

IMP name	BNT165e, consisting of the following components: • BNT165c • BNT165d (a 1:1 mixture of BNT165d1 and BNT165d2). Isotonic NaCl solution (0.9%) will be used as placebo in Part A.
Type	Investigational
Route of administration	IM injection in the deltoid muscle of the non-dominant arm. Other injection sites may be used if necessary.
Dose levels	Part A: Refer to Table 1.
Vaccination schedules	Part A: Three (one each on Days 1, 57, and 183) injections given to Cohorts 1 to 9. Three (one each on Day 1, Day 29 and Day 57) injections given to Cohort 10.
Packaging and labeling	IMP will be provided in glass vials. Each vial will be labeled as per country requirements. See the Pharmacy Manual for guidance for the preparation of the IMP.

Abbreviations: IM = intramuscular; IMP = investigational medicinal product.

6.2 Dosing and administration

Subjects in Cohorts 1 to 9 of Part A will receive 3 doses of BNT165e (or its components) on Day 1, Day 57 and Day 183 as depicted in the SoA (Section 1.3). Subjects in Cohort 10 will receive 3 doses of BNT165e on Day 1, Day 29 and Day 57 (Section 1.3). IMP should be administered intramuscularly into the deltoid muscle, preferably of the non-dominant arm.

Standard vaccination practices must be observed. Appropriate medications and supportive measures for the management of acute anaphylactic reactions need to be available on site according to local guidelines. Administration of IMP should be performed by an appropriately qualified, Good Clinical Practice (GCP)-trained, and vaccine-experienced member of the trial staff as allowed by local, state, and institutional guidelines. Please refer to Section 6.6.2.2 for information on blind status of IMP administrators.

6.2.1 Dose modifications

The IRC may request reduction of the planned dose in any cohort.

6.3 Rationale for the trial treatment

The proposed dose levels for BNT165e and its components in this trial are based on safety and immunogenicity data from non-clinical studies and on the safety data generated for other members of the same RNA technology platform such as the SARS-CoV-2 vaccine BNT162b2 licensed as “COMIRNATY” and given emergency use authorization as “Pfizer-BioNTech COVID-19 vaccine”. BNT162b2 and the BNT165e have the same LNP carrier system, the same nucleoside-modified RNA, and the same untranslated elements of the antigen-encoding RNA (the RNAs differ essentially in the encoded open reading frames for antigen expression).

The doses of BNT165e vaccine components BNT165c (10 µg) and BNT165d (30 µg) tested initially during the dose-escalation phase in Part A of this trial are comparable to the

dose approved for BNT162b2 (30 µg). The vaccine doses used in this trial are also spread over more time; the primary two doses in the BNT162b2 trials were given 3 weeks apart, but in this trial the initial two doses will be administered ~8 weeks (~57 d) apart in Cohorts 1 to 9 and the third dose will be administered ~18 weeks after the second dose (~183 d after the first dose) in Cohorts 1 to 9. In Cohort 10, IMP is administered 28 d apart on Day 1, Day 29 and Day 57 (Section 1.3).

The highest dose level for BNT165e in this trial (100 µg) is covered by the existing toxicology platform data on BNT162 vaccines that evaluated doses up to 100 µg in rats. Also, in the SARS-CoV-2 BNT162 Phase I trial BNT162-01 (NCT04380701), doses of 50 µg BNT162b1 given twice 3 weeks apart were evaluated in humans and no SAEs related to the vaccine were observed, while reactogenicity showed clear dose level dependency. To fully explore the immunogenicity dose-response curve and tolerability of BNT165e in this first-in-human (FIH) trial, different doses of BNT165c and BNT165d will be tested, up to a maximum combined dose of 100 µg.

Since its first marketing authorization in December 2020, over 4.3 billion doses of the original and bivalent BNT162b2 vaccines had been shipped worldwide to more than 100 countries globally by 18 DEC 2022. More than 2.5 billion doses are estimated to have been administered. Notably, these vaccines have been administered safely in pediatric, pregnant and immunocompromised populations, i.e., populations that are relevant to BNT165e development since these groups are at increased risk of severe malaria. Pharmacovigilance data on BNT162b2 continues to accumulate, and to date, continues to support a favorable safety profile with no specific safety concerns for the prevention of COVID-19 disease.

For the above reasons, the planned trial treatments in this trial are considered to be justified.

6.4 Actions in case of overdose or errors in drug administration

For a definition of an overdose, see the subsection “AEs associated with an overdose or error in drug administration” in Section 10.4.1.3.3.

The sponsor does not recommend specific treatment for an overdose.

In the event of an overdose or an error in drug administration, the investigator should:

- Contact the trial medical monitor immediately.
- (At the discretion of the investigator) Give symptomatic treatment.
- Closely monitor the trial subject for any AE/SAE and laboratory abnormalities (at least 7 d).
- Document the quantity of the excess dose.

6.5 Preparation/handling/storage/accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all trial treatment received and any discrepancies are reported and resolved before use of the trial treatment.

Only trial subjects enrolled in the trial may receive trial treatment and only authorized site personnel may handle, prepare and administer trial treatment. All trial treatment must be

stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to authorized pharmacy or site personnel (unblinded personnel Part A).

The investigator, site, or the head of the site (where applicable) is responsible for trial treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Any non-compliance with the provided storage conditions should be reported to the sponsor upon discovery along with any actions taken. Once non-compliance is identified, the trial treatment must be quarantined and not used until the sponsor provides permission to use/discard/return the trial treatment.

Further guidance and information for the storage and final disposition of unused trial treatment are provided in the Pharmacy Manual.

6.6 Subject assignment, randomization, and blinding

6.6.1 Randomization

In Part A, one cohort will be opened at a time. Subjects will be randomized in a 5:1 ratio to either active IMP or placebo.

Randomization will be performed using an Interactive Web Response System with a central randomization procedure. A computer-generated randomization schedule will be created by an external vendor.

6.6.2 Blinding/Unblinding

Part A of this trial is observer-blind.

6.6.2.1 Blinding of trial subjects

In Part A, all randomized trial subjects will be blinded to their randomized trial treatments.

6.6.2.2 Blinding of trial site personnel

In the observer-blinded Part A of this trial, the trial site personnel receiving, storing, dispensing, preparing, and administering the trial IMP will be unblinded. All other trial site personnel, including the investigator and investigator staff, will be blinded to trial IMP assignments. In particular, the individuals who evaluate subject safety will be blinded. Because there are differences in physical appearance of the trial IMPs, the trial treatment will be administered in a manner that maintains the blinding, e.g., syringes will be masked or colored. For details, see the Pharmacy Manual.

The investigator will assign the responsibility of the unblinded dispensers/administrators to persons who will not participate in the evaluation of any trial subject. To ensure adequate coverage, at least two unblinded dispensers/administrators will be assigned per trial site. Members of the trial site personnel or clinic pharmacy should fulfill these roles. Contact between the unblinded dispensers and trial subjects should be kept to a minimum. The investigator and trial site personnel other than the unblinded dispensers/administrators must not be allowed to know the IMP assigned to any trial subject and must not be allowed to see the IMP container contents. In the event of a Quality Assurance audit, the auditor(s) will be allowed access to unblinded trial IMP records at the site(s) to verify that randomization/dispensing has been done accurately.

6.6.2.3 Blinding of sponsor personnel

To facilitate rapid review of data in real time, sponsor staff will be unblinded to trial intervention allocation. However, sponsor staff who routinely have contact with blinded site personnel, and who are not involved in ensuring that protocol requirements for trial treatment preparation, handling, allocation, and administration are fulfilled at the site, will also remain blinded on a subject-level. Further details will be specified in a Blind Management Plan.

6.6.2.4 Blinding of laboratory personnel

In this observer-blind trial, laboratory personnel performing genetic (see Section 8.5) and immune response assays (see Section 8.6) will be blinded. However, sponsor laboratory personnel will be unblinded.

6.6.2.5 Unblinding

In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a trial subject's trial treatment assignment is warranted. Trial subject safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the trial medical monitor before unblinding a trial subject's trial treatment assignment unless this could delay further management of the trial subject. If a subject's trial treatment assignment is unblinded, the sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and case report form (CRF). Decision on trial continuation of unblinded trial subjects will be made between investigator and trial medical monitor on a case by case basis.

6.7 Trial treatment adherence

Trial treatment will be administered directly from an unblinded designee of the investigator (Part A). The date and time of each dose administered must be recorded. The subject identification number will be confirmed at the time of dosing by a member of the trial site personnel other than the person administering the trial treatment.

See Section 6.4 for guidance on the treatment of overdose or errors in drug administration.

6.8 Access to trial treatment after the end of the trial

Provision of access to trial treatment after the end of the trial is not planned.

6.9 Concomitant therapy

6.9.1 Prohibited medication during the trial (if applicable)

Receipt of the following vaccines and medications during the time periods listed below may exclude a subject from a per-protocol perspective from that point onward and may require trial vaccinations to be discontinued in that subject-upon discussion of the investigator and the trial medical monitor. Medications should not be withheld from a subject's medical care.

Trial subjects should not receive any non-trial vaccinations starting at Visit 0 and continuously until 28 d after Dose 3 for Part A in this trial unless medically indicated. See exclusion criterion 10 (Part A).

Trial subjects should not receive blood/plasma products or Ig from 120 d before Dose 1 and continuously until the last trial visit (365 d after Dose 3). See exclusion criterion 11 (Part A).

Trial subjects should not receive any allergy treatment with antigen injections within 28 d before each IMP administration for Part A. See exclusion criterion 12 (Part A).

Trial subjects should not receive any immunosuppressive therapy, including systemic corticosteroids (if systemic corticosteroids are administered for ≥14 d at a dose of ≥20 mg/d of prednisone or equivalent) starting at Visit 0 and continuously until 28 d after Dose 3 for Part A. Intraarticular, intrabursal, or topical (skin or eyes) corticosteroids are permitted. See exclusion criterion 13 (Part A).

Trial subjects should not receive prescription or nonprescription drugs (excluding vitamins and dietary or herbal supplements) starting at Visit 1 and continuously until 28 d after Dose 3 for Part A, unless they are considered permitted medications (described in the next section) or medically indicated and that in the opinion of the investigator and sponsor will not interfere with the trial.

6.9.2 Permitted medication during the trial (if applicable)

Trial subjects should not receive prophylactic antipyretics and other pain medication to prevent symptoms associated with IMP administration. However, if a trial subject is taking a medication for another condition, even if it may have antipyretic or pain-relieving properties, it should not be withheld before IMP administration in this trial.

Inhaled, topical, or localized injections of corticosteroids (e.g., intraarticular or intrabursal administration) are permitted.

Administration of standard therapeutic dose of acetaminophen (up to 4 g/d) is permitted. If acetaminophen is contraindicated, then a non-steroidal anti-inflammatory drug (NSAID) is permitted.

For intercurrent infections e.g., urinary tract infections and sinusitis, a short course (up to 7 d) of oral antibiotics are permitted during the trial.

Other concomitant medication may be considered on a case by case basis by the investigator, if required after consultation with the trial medical monitor.

6.9.3 Recording concomitant medication during the trial

All concomitant medication permitted or prohibited (including over the counter or prescription medicines, vitamins, and/or herbal supplements) that the trial subject receives during the trial must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The sponsor’s medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

The start and stop dates and name of the concomitant medication during the trial will be recorded for the period defined in the SoA (Section 1.3).

6.10 Treatment for specific adverse reactions

There are no AEs that are expected for the candidate vaccines as this is the FIH trial. Anticipated adverse reactions are mostly reflective of mild to moderate local and systemic reactogenicity events. Additionally, anaphylaxis can occur after any vaccination. For further details see the BNT165e IB.

6.10.1 Myopericarditis

Any subject who reports acute chest pain, shortness of breath, palpitations, or any other symptom(s) that might be indicative of myocarditis or pericarditis must be specifically evaluated by a cardiologist for possible myocarditis or pericarditis. Subjects will be instructed to immediately contact the investigator in case any of these symptoms occurs. The same applies for any subject in whom a new ECG abnormality (compared to baseline) consistent with probable or possible myocarditis or pericarditis or abnormal troponin I or T level is observed.

In addition to a clinical evaluation, the following should be performed:

- ECG and
- Measurement of the troponin I or T level

If myocarditis or pericarditis is suspected based upon the initial evaluation, the following should also be performed:

- Cardiac echocardiogram and/or
- Cardiac magnetic resonance study

Any case of myocarditis, pericarditis or myopericarditis, that is assessed as possibly related by the investigator will constitute a trial treatment pausing rule per Section 7.1.1, until the IRC and the IDMC independently review the event and provide a recommendation. The ECG abnormalities consistent with probable or possible myocarditis or pericarditis are those judged as such by a cardiologist, including:

- Sustained atrial or ventricular arrhythmias
- Second-degree Mobitz Type II or worse atrioventricular block, new bundle branch block
- Diffuse ST-segment elevation or PR-segment depression, compatible with pericarditis

The investigators must follow-up on any case of clinically significant ECG abnormality with relevant tests till resolution.

6.10.2 Other specific reactions

Treatment reflective of mild to moderate local and systemic reactions is at the discretion of the investigators; the following suggestions are provided:

- After the first occurrence of systemic symptomatology including fever, subjects can be treated with standard therapeutic dose of acetaminophen up to 4 g/day (preferable), or ibuprofen up to 1,200 mg/day if acetaminophen is contraindicated.
- Ensure adequate hydration of trial subjects on the day of vaccination, e.g., by asking trial subjects to drink water before each site visit and during the ~2 h after each IMP administration per trial site standard.

Expected adverse reactions are mostly reflective of mild to moderate local and systemic reactogenicity events. Additionally, anaphylaxis can occur after any vaccination. For further details see the BNT165e IB.

7 DISCONTINUATION OF TRIAL TREATMENT AND TRIAL SUBJECT DISCONTINUATION/WITHDRAWAL

Any safety concerns of the investigators should be discussed with the sponsor immediately upon occurrence or awareness to determine if the trial subject should continue or discontinue trial treatment.

7.1 Discontinuation or pausing (temporary halting) of trial treatment

The investigator shall notify the sponsor within 24 h about any observed Grade 3 AE (Section 7.1.1) or above that is related on an expedited timeline like that of an SAE. A paper form to collect SAEs and Grade 3 or 4 AEs contributing to pausing rules will be used for collecting these events. Grade 3 reactogenicity events are not reported on a paper SAE form unless one continues on Day 7 post-IMP dose. If a Grade 3 or worse reactogenicity event continues on Day 7 post-IMP dose, it will be reported as an AE and needs to be reported on a paper SAE form.

If the reported events fulfill pausing rules criteria:

- The IRC will review all related data.
- Randomization and trial treatment administration for the impacted cohort and any cohort that has a higher BNT165c or BNT165d combined dose will be paused until the IRC recommends that assignment to the cohort(s) and administration of trial treatment may be resumed.

For all subjects who are vaccinated, all other routine trial conduct activities, including ongoing data entry, reporting of AEs, subject reactogenicity e-diary completion, blood sample collection, and subject follow-up, will continue during the pause up to but not including the visit of the next dose. In case trial treatment is permanently discontinued, the subjects who have not completed all doses will be followed-up as specified in Section 7.1.3.

7.1.1 Cohort trial treatment pausing rules

Grading scales for the intensity of AEs and SAEs will be based on guidance from the US FDA (US FDA 2007).

If any of the below is met, no additional subjects will be assigned to the trial and administration of IMP for all cohorts, including administration of Dose 2 and Dose 3 for all cohorts, will be paused until the IRC reviews the event(s):

- Any SAE developed by a subject administered with trial treatment (at any dose combination), for which there is no alternative, plausible, attributable cause and which is assessed by the investigator, or the sponsor as related.
- Any severe (Grade 3) hypersensitivity or any anaphylactic AE that occurs within 7 d of vaccination without a clear alternative cause.

NOTE: Grade 3 hypersensitivity is defined as symptomatic bronchospasm with or without urticaria, parenteral intervention, allergy related edema/angioedema, or hypotension. Grade 4 hypersensitivity encompasses all life-threatening events and for which urgent intervention is indicated.

- Any Grade 4 AE after administration of trial treatment (at any dose combination) that is assessed as related by the investigator, or for which there is no alternative, plausible, attributable cause or any Grade 4 reactogenicity event.
- Any AE that may be an immune-mediated disease, including but not limited to myocarditis, pericarditis or myopericarditis or other significant immune-mediated condition that may result in end-organ damage, be life-threatening, or otherwise result in significant downstream health consequences that is assessed as related by the investigator, or for which there is no alternative, plausible, attributable cause.
- Any case of fever >40.0°C (measured orally) persisting for over 6 h despite the use of antipyretics after administration of trial treatment (at any dose combination) that is assessed as related by the investigator and/or sponsor, or for which there is no alternative, plausible, attributable cause.
- If two trial subjects develop a similar Grade 3 AE at least possibly related to trial treatment (unsolicited AEs, and laboratory abnormalities). Grade 3 reactogenicity events reported within 7 d from the day of administration of IMP as recorded by e-diary are excluded from this pausing rule.

NOTE:

- Reactogenicity e-diary data confirmed by the investigator as being entered by the subject in error will not contribute toward a pausing rule.
- Data from placebo recipients will not support the pausing rules.

7.1.2 Individual trial subject trial treatment pausing rules

Trial treatment administration for an individual trial subject will be paused (if applicable), pending IRC review, if any of the below criteria are met:

- Safety concerns identified by an investigator or the IRC
- Subject request for medical reasons (e.g., severe local reaction)

A febrile illness (body temperature ≥38.0°C/≥100.4°F) or other acute illness within 48 h before any IMP administration is a reason for temporary deferral of a dose, until fever-free for at least 24 h without antipyretics.

7.1.3 Permanent discontinuation of single trial subjects from vaccination

If the following occur, a single trial subject will be discontinued from further doses of trial vaccine:

- SAE assessed by the Investigator as related to trial treatment.
- Grade 3 or higher unsolicited event assessed by the investigator as related to trial treatment.

Other reasons for permanent discontinuation of vaccination include the following: AEs, subject request, investigator request, pregnancy, and protocol deviation (including no longer meeting all of the inclusion criteria or meeting one or more exclusion criteria).

Note that discontinuation of vaccination does not represent withdrawal from the trial. If vaccination is permanently discontinued, the subject should remain in the trial to be evaluated for safety and immunogenicity. See the SoA (Section 1.3) for data to be collected at the time of discontinuation of vaccination and follow-up for any further evaluations that need to be completed.

All subjects that are early terminated from treatment but not from the trial should undergo regularly scheduled activities through 28 d post last received IMP dose, per SoA (Section 1.3). After this point, subjects that are discontinued from trial treatment after Dose 1 should receive a safety phone call at 6 months (±14 d) and 12 months (±14 d) post last received IMP dose. Subjects that are discontinued from trial treatment after Dose 2 should perform all visits and assessments per protocol, except for IMP-related procedures. The site investigator will receive a guidance document detailing the applicable assessments of treatment-discontinued subjects and can consult the trial Medical Monitor in case of uncertainty. In cases of pregnancy and other instances where further blood draws may be medically contraindicated in the opinion of the site investigator, no blood draws will be performed during the follow-up.

In the event of discontinuation of vaccination, it must be documented whether the subject is discontinuing further receipt of vaccination or also from trial procedures, post-vaccination trial follow-up, and/or future collection of additional information.

Subjects with events of myocarditis and/or pericarditis should be discontinued from further vaccination; however, they should continue, at a minimum, to be followed for safety as described in this section and also in Section 6.10.1.

7.1.4 Criteria for temporarily delaying enrollment, randomization or administration of IMP

Enrollment or randomization into the dosing cohorts may be paused or delayed if the IRC recommends a pausing of trial treatment according to Section 7.1.1. Trial subjects in Cohorts 1 to 9 in Part A may receive Dose 3 of IMP up to 30 d after Day 183 (extended Visit 13 window -7 d/+30 d) in the setting of a temporary trial pause that becomes lifted. The dosing administration window for Dose 2 of IMP is not extended in the setting of a trial pause.

7.2 Trial subject discontinuation or withdrawal from the trial

A subject may withdraw from the trial at any time at their own request. Reasons for discontinuation from the trial include the following:

- Refused further trial procedures;
- Lost to follow-up;
- Death;
- Trial terminated by sponsor;
- AEs;
- Subject request;
- Investigator request.

The investigator or his or her designee should capture the reason for discontinuation/withdrawal for all subjects. If a subject withdraws from the trial, they may request destruction of any remaining samples taken and not tested, and the investigator must document any such requests in the site trial records and notify the sponsor accordingly. If the subject withdraws from the trial and also withdraws consent (see Section 10.1.4 for disclosure of future information), no further evaluations will be performed, and no additional data will be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

Unless the subject withdraws consent to participate in the trial every effort will be made to follow subjects for safety assessments. If trial subjects are discontinued from further vaccination they should be followed-up as detailed in Section 7.1.3.

7.2.1 Withdrawal of consent

Subjects who request to discontinue receipt of vaccination will remain in the trial as described in Section 7.1.3. The only exception to this is when a subject specifically withdraws consent for any further contact with them or persons previously authorized by the subject to provide this information. The withdrawal of consent should be explained in detail in the medical records by the investigator, as to whether the withdrawal is only from further receipt of vaccination or also from trial procedures and/or post-vaccination trial follow-up.

7.3 Lost to follow-up

A trial subject will be considered lost to follow-up if they repeatedly fail to return for scheduled visits and are unable to be contacted by the trial site.

The following actions must be taken if a trial subject fails to return to the trial site for a required trial visit:

- The trial site must attempt to contact the trial subject and reschedule the missed visit as soon as possible and counsel the trial subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the trial subject wishes to continue in the trial.

- Before a trial subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the trial subject (where possible, three telephone calls and, if necessary, a certified letter to the trial subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the trial subject's medical record.
- If the trial subject continues to be unreachable, they will be considered to have withdrawn from the trial.

7.4 Replacement of subjects discontinued from the trial or further vaccination

Subjects who are out of window for dosing in the event of a trial pause can be discontinued from further vaccination and followed up for safety per Section 7.2. In settings where a subject is discontinued either from further vaccination or discontinued from the trial, the sponsor can decide to enroll an additional subject in the cohort to replace the discontinued subject, even in the setting that they will still be followed for safety.

8 TRIAL ASSESSMENTS AND PROCEDURES

The investigator (or an appropriate delegate at the investigator site) must obtain a signed and dated informed consent before performing any trial-specific procedures.

All screening evaluations must be completed and reviewed to confirm that a potential trial subject meets all eligibility criteria. The investigator will maintain a screening log to record details of all volunteers screened and to confirm eligibility or record reasons for screening failure, as applicable.

Protocol waivers or exemptions are not allowed.

See the SoA (Section 1.3) for all planned time points for assessments.

Any safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the trial subject should continue or discontinue trial treatment.

Adherence to the trial design requirements, including those specified in the SoA, is essential and required for trial conduct, but there may be protocol deviations. Every effort should be made to ensure that protocol-required activities are completed as described. However, it is anticipated that from time to time there may be circumstances outside the control of the investigator that may make it unfeasible to perform the planned activity. In these cases, the investigator must take all steps necessary to ensure the safety and wellbeing of the trial subject. When a protocol-required activity cannot be performed, the investigator must document the reason for the missed activity and any corrective and preventive actions taken to ensure that required processes are adhered to as soon as possible. The sponsor must be informed of these incidents in a timely manner.

For samples being collected and shipped, detailed collection, processing, storage, and shipment instructions and contact information will be provided to the investigator site before initiation of the trial.

Procedures conducted as part of the trial subject's routine clinical management (e.g., blood count) and obtained before signing of the informed consent may be utilized for

screening or baseline purposes provided the procedures met the protocol specified criteria and were performed within the time frame defined in the SoA (Section 1.3).

8.1 Screening/baseline assessments and procedures

At screening, the following demographic data and measurements will be recorded for all trial subjects:

- Age
- Sex
- Ethnic group
- Race
- Body weight, height, and derived body mass index

Medical history information will be recorded at the times given in the SoA (Section 1.3).

8.2 Efficacy assessments

Not applicable.

8.3 Safety assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.3).

8.3.1 Physical examinations

Physical examinations will be performed by the investigator or a designee with a medical license at the trial site according to the SoA (Section 1.3).

- A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal and neurological systems.
- An abbreviated physical examination (a limited, symptom-directed physical examination) will be performed at the other times noted in the SoA, if clinically indicated. New or worsened clinically significant abnormalities will be recorded on the AE page of the CRF.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

Any untoward physical examination findings that are identified during the active collection period and meet the definition of an AE or SAE (see Sections 10.4.1 and 10.4.2) must be reported according to the processes in Sections 10.4.3 and 10.4.4.

8.3.2 Vital signs, height, and body weight

Vital signs (oral body temperature, systolic/diastolic blood pressure, pulse rate, and respiratory rate) will be assessed at the time points listed in Section 1.3.

The same position and methods for measuring the vital signs should be used for one trial subject throughout the trial.

Vital signs measurements should be preceded by at least 5 minutes of rest for the trial subject in a quiet setting without distractions (e.g., television, cell phones). Vital signs will be measured with trial subjects in the seated position and before any blood draws.

Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

If vital signs measurements are abnormal, two further measurements must be done and the average of the three measurements must be recorded on the CRF).

Height and body weight will be measured at the times given in the SoA. Assessment of body weight should be repeated at any time if there are apparent weight changes.

8.3.3 Electrocardiograms

Planned time points for 12-lead ECGs are provided in the SoA (Section 1.3).

Standard 12-lead ECG (using limb leads and with a 10-second rhythm strip) will be recorded as outlined in the SoA using an ECG machine that automatically calculates the heart rate and measures PR interval, QRS complex, QT interval, and QTcF.

All ECG recordings should be preceded by at least 10 minutes of rest for the trial subject in a quiet setting without distractions (e.g., television, cell phones). All ECGs should be performed with subjects in a supine position. All ECGs should be obtained before blood collection, measurement of vital signs, and IMP administration.

For Part A:

- The ECG needs to be manually assessed by the investigator. The manual assessment (not the automated machine assessment) will be the final interpretation of the ECG recording.
- The ECG and the report need to be maintained in the subject's source documentation.
- Only ECG readings that are found by the investigator or their designee to be abnormal and clinically significant will be reported as an AE in the CRF.
- The investigator or their designee is responsible for assessing clinical significance of any ECG finding in the CRF.

In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality. It is important that lead placement be in the same position each time to achieve precise ECG recordings.

Any untoward findings that are identified during the active collection period and meet the definition of an AE or SAE (see Sections 10.4.1 and 10.4.2) must be reported according to the processes in Sections 10.4.3 and 10.4.4.

ECG findings of potential clinical concern are listed in Section 8.3.3.1. ECG findings consistent with probable or possible myocarditis or pericarditis are defined in Section 6.10.1.

8.3.3.1 Guidance for ECG findings of potential clinical concern

Appendix 1 contains a list of potential clinically significant ECG findings/events but is not an all-inclusive list of what should be reported as AE/SAEs.

8.3.4 Clinical laboratory tests

Planned time points for biosampling for clinical laboratory tests are provided in the SoA (Section 1.3).

The below listed clinical laboratory parameters will be assessed by a central laboratory except the urine pregnancy test, which will be performed locally. Blood sampling for additional clinical laboratory tests (including the addition of parameters) may be performed at any time during the trial as determined necessary by the investigator or required by local regulations.

The planned clinical laboratory parameters include:

- Chemistry: alkaline phosphatase, alanine transaminase, aspartate transaminase, total bilirubin, blood urea nitrogen, creatinine, troponin I (if troponin I is unavailable troponin T can be used). Only in volunteers born female where the postmenopausal status needs to be confirmed; follicle stimulating hormone (FSH) at screening.
- Hematology: HbA1c (only screening HbA1c will be collected), hemoglobin, hematocrit, red blood cell count, white blood cell count and differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelet count.
- Only for VOCBP: serum β-HCG at Visit 0 and urine pregnancy test on the day of dosing before IMP administration.

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the trial as AEs in the AE section of the CRF. The laboratory reports must be filed with the source documents. For a definition of clinically significant abnormal laboratory findings, see Section 10.4.1.3.2.

All laboratory tests with values considered clinically significantly abnormal within 30 d after the last dose of trial treatment should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.

If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.

If laboratory values from non-protocol specified laboratory assessments performed at the site's local laboratory require a change in trial subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification), then this must be recorded in the applicable section of the CRF.

Any (serious) AEs resulting from laboratory tests values will be graded by the investigator.

For further guidance refer to the US FDA guidance (US FDA 2007).

8.3.5 Viral screening

Viral screens will be performed at the visit given in the SoA (Section 1.3). The viral screen comprises a screen for HIV 1, HIV 2, Hepatitis B, and Hepatitis C. Viral screen testing will be performed at a central laboratory.

8.3.6 Subject e-diaries for assessment of reactogenicity (local and systemic reactions)

Subject e-diaries will be issued, trained, and collected by trial site personnel at the visits given in the SoA (Section 1.3).

Subjects will be asked to:

- Report reactogenicity (incl. oral body temperature) daily for 7 d after each IMP dose using an e-diary.
- Report the use of medications to treat symptoms associated with IMP administration for 7 d after each IMP dose using an e-diary.
- Record the worst grade for each symptom at approximately the same time every evening.
- Contact the site if they experience any severe or potentially life-threatening reactogenicity events.

Trial subjects will be trained by the site personnel for the completion of the e-diary using the training material provided by the sponsor. Moreover, the subjects will receive the e-diary participant guide with step-by-step instructions.

The trial site personnel will remind the trial subject to record the worst grade for each symptom in the e-diary at approximately the same time every evening starting on the day of IMP injection and then every day in the evening for a total of seven consecutive days.

The trial site personnel will remind the trial subject to measure their oral body temperature using the provided device and record it in the e-diary every day including the day of IMP injection for a total of seven consecutive days. The e-diary system sends automated e-mail alerts to trial sites of affected subjects and sponsor for any missed entries. The trial site personnel will contact subjects for any missed e-diary entry.

The grading of reported solicited local and systemic reactions will be performed using the grading scale tables given in Sections 8.3.6.1 and 8.3.6.2, which are from the FDA Guidance for Industry (US FDA 2007). Confirmation by an investigator or medically qualified person is required for all Grade 3 or 4 reactogenicity events.

Grade 4 reactogenicity events will be documented in an electronic data capture (EDC) system only and not in the subject's e-diary.

8.3.6.1 Assessments of intensity for local reactions

During the reactogenicity e-diary reporting period, subjects will be asked to assess erythema/redness, induration/swelling and pain at the injection site and to record the symptoms in the reactogenicity e-diary. If a local reaction persists beyond the end of the reactogenicity e-diary period following vaccination, the subject will be requested to report that information. The investigator will enter this additional information in the EDC system.

Redness and swelling/induration will be measured and recorded in centimeters or inches and then categorized during analysis as absent, mild, moderate, or severe according to the grading scale in Table 4. Pain at the injection site will also be assessed by the subject as absent, mild, moderate, or severe according to the grading scale in Table 4.

If a Grade 3 local reaction is reported in the reactogenicity e-diary, an automated e-mail alert will be sent to the site and the sponsor. The subject will be contacted by the

investigator or a medically qualified person by phone to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person will be able to classify a subject's local reaction as Grade 3 or 4 and confirm in the CRF.

Table 4: Local reaction grading scale

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

a Investigator or medically qualified person confirmation is required for Grade 3 or 4. Grade 4 reactions will be documented in the EDC system only and not the subject's e-diary.

Abbreviation: EDC = electronic data capture.

Source: Modified from the US FDA guidance (US FDA 2007).

8.3.6.2 Assessments of intensity for systemic reactions

During the reactogenicity e-diary reporting period, subjects will be asked to assess vomiting, diarrhea, headache, fatigue/tiredness, muscle pain/joint pain and to record the symptoms in the reactogenicity e-diary. The symptoms will be assessed by the subject as absent, mild, moderate, or severe according to the grading scale in Table 5.

If a Grade 3 systemic reaction is reported in the reactogenicity e-diary, an automated e-mail alert will be sent to the site and the sponsor. The subject will be contacted by the investigator or a medically qualified person by phone to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person will be able to classify a subject's systemic reaction as Grade 3 or 4 and confirm in the CRF.

Temperature will be recorded in the e-diary during the e-diary reporting period. If a fever of ≥39.0°C (≥102.1°F) is reported in the reactogenicity e-diary, an automated e-mail alert will be sent to the site and the sponsor. The subject will be contacted by the investigator or a medically qualified person by phone to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person will be able to confirm a subject's fever as >40.0°C (>104.0°F).

Table 5: Systemic reaction grading scale

	Mild (Grade 1)	**Moderate (Grade 2)**	**Severe (Grade 3) [a]**	**Potentially life-threatening (Grade 4) [a]**
Fever (oral temperature of ≥38.0°C/≥100.4°F)	38.0°C – 38.4°C 100.4°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	39.0°C – 40.0°C 102.1°F – 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
Muscle/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 muscle/joint pain

a Investigator or medically qualified person confirmation is required for Grade 3 or 4. Grade 4 reactions (except fever) will be documented in the EDC system only and not the subject's e-diary.

Abbreviation: EDC = electronic data capture.

Source: Modified from the US FDA guidance (US FDA 2007).

8.3.7 Injection site reactogenicity assessments

Investigators will assess the injection site for reactogenicity symptoms at the visits and times given in the SoA (Section 1.3).

Investigators will grade and record any observed reactogenicity symptoms as described in Section 8.3.6.1. If any local reactions meet any SAE criteria, they must be recorded as an SAE.

8.3.8 Subject monitoring after each IMP dose

Subjects will be monitored by trial site personnel for adverse reactions for at least 1 hour after each IMP dose.

8.3.9 Subject hotline

Trial subjects will be provided with contact details for a trial site Subject hotline, which can be used to contact the trial site during their participation in the trial should they require guidance or should they experience any symptoms of illness. The reporting of any symptoms of illness, e.g., flu-like symptoms, may trigger diagnostic measures (including *ad hoc* site visits) at the discretion of the investigator.

For guidance on the handling of specific adverse reactions, see Section 6.10.

8.3.10 Subject phone calls

Subjects will be contacted for non-leading phone calls at the times given in the SoA (Section 1.3). During the 14 d post-IMP administration phone call, subjects will specifically be asked about potential delayed local injection site reactions. During this phone call, they will be asked to report on any new skin reaction at the site of injection in the last 7 d before the phone call (Day 8 to Day 14 post-IMP administration), which will be recorded as an AE.

8.4 Adverse events and serious adverse events

Definitions of AEs and SAEs can be found in Section 10.4.

8.4.1 Time period and frequency for collecting AE and SAE information

All AE and SAE information will be collected at the time points specified in the SoA (Section 1.3).

Non-serious AEs will only be collected as of first IMP dose administered while SAEs need to be recorded upon awareness starting once informed consent is given.

Adverse events will be collected until 28 d after Dose 3. Medically attended adverse events (MAAEs) and SAEs need to be recorded continuously until the end of the trial (365 d after Dose 3). If vaccination is permanently discontinued, unsolicited AEs will be followed for 28 d following the last IMP dose. In the trial subjects for whom vaccination is permanently discontinued, MAAEs and SAEs should be collected for at least 365 d post last received IMP dose (see Section 7.1.3 for specifics about follow-up).

Medical occurrences that begin after obtaining informed consent but before the start of trial treatment administration must be recorded on the respective Medical History/Current Medical Conditions section of the CRF and not in the AE section unless they qualify as SAEs or occurrences that are clearly related to the trial's procedures/assessments.

Medical occurrences meeting the criteria for an SAE (see Section 10.4.2) must be reported as SAEs (see Section 8.4.4).

8.4.2 Detecting and reporting AEs and SAEs

The investigator and any qualified designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up all AEs and SAEs. The investigator will record on the CRF all directly observed and all spontaneously reported AEs and SAEs reported by the trial subject.

Open-ended and non-leading verbal questioning of the trial subject is the preferred method to inquire about AE occurrences. Care will be taken not to introduce bias when detecting AEs and/or SAEs.

The recording, evaluating, and assessing of AEs and SAEs are provided in Section 10.4.

For grading the severity of AEs and SAEs, the FDA Guidance for Industry (US FDA 2007) will be used to grade the severity of AEs and SAEs.

Investigators are not obligated to actively seek AE or SAE after conclusion of the trial participation. However, if the investigator learns of any SAE, including a death, at any time after a trial subject has been discharged from the trial, and they consider the event to be related (see Section 10.4) to the trial treatment or trial participation, the investigator must promptly notify the sponsor as described in Section 10.4.4.

The investigator may be requested by the sponsor to obtain specific follow-up information in an expedited fashion.

Prompt notification of SAEs by the investigator to the sponsor is essential so that the ethical responsibilities to trial subjects, the safety of a trial treatment under clinical investigation, and regulatory reporting obligations can be met.

For real time monitoring of AEs which may contribute to pausing rules criteria, all such AEs (Grade 3 or 4, which are confirmed and assessed by the investigator as related and/or for which there is no alternative, plausible, attributable cause) need to be reported on an expedited timeline (within 24 hours) as done for SAEs.

All SAEs and Grade 3 or 4 AEs which may contribute to pausing rules (initial and follow-up reports) will be recorded and reported via paper form to the sponsor or designee within 24 hours after the site becoming aware of the event, as indicated in Section 10.4.4.

8.4.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each trial subject at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the trial subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Section 10.4.2.2.

For subjects who are screen failures, the SAE collection ends when screen failure status is determined.

If the subject withdraws informed consent, the AE collection ends when consent is withdrawn.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

If a trial subject dies during participation in the trial (see the SoA in Section 1.3), the investigator will provide the sponsor a copy of any postmortem findings including histopathology if available.

Follow-up information on SAEs and Grade 3 or 4 AEs which contribute to pausing rules will be recorded in the CRF and also reported via paper form. These follow-up reports will be sent to the sponsor or designee within 24 h after the site becomes aware of the event, as indicated in Section 10.4.4.

8.4.4 Regulatory reporting requirements for SAEs including SUSARs

Prompt notification of an SAE via the provided paper SAE form by the investigator to the sponsor within 24 hours of the site's awareness is essential so that the ethical responsibilities to trial subjects, the safety of a trial treatment under clinical investigation, and regulatory reporting obligations can be met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies involved in the conduct of trials with the same IMP about the safety of a trial treatment under clinical investigation. The sponsor will comply with country-specific

regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC) as applicable, and investigators. The execution of reporting to the different entities may be delegated as detailed in the trial-specific Safety Management Plan.

All serious adverse reactions, the nature, severity or outcome of which is not consistent with the reference safety information are “unexpected serious adverse reaction”. The expectedness assessment for all related SAEs is based on the reference safety information included in Section 6.1.5 of the BNT165e IB.

All suspected adverse reactions related to an IMP that occur in this trial, and that are both unexpected and serious are qualify as SUSARs. SUSARs are subject to expedited reporting requirements according to applicable regulatory requirements and guidance (e.g., ICH E2A guidance).

For the IMPs, it is the sponsor’s or delegate’s responsibility to perform expedited SUSAR reporting submission to the applicable regulatory authorities, Institutional Review Boards/Independent Ethics Committees (IRB/IECs) within the timelines stipulated in the respective country regulations. Reporting to the investigators will follow country-specific regulatory requirements and applicable guidelines.

An investigator who receives an investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) or SUSARs from the sponsor should review it and then file it together with the IB. If required by local requirements, the investigator will notify the relevant IRB/IEC.

8.4.5 Pregnancy counseling, testing, and collection of pregnancy information

For VOCBP, pregnancy tests and counseling will be performed at the times given in the SoA (Section 1.3).

Any trial subject who becomes pregnant while participating in the trial will be immediately permanently discontinued from trial treatment (see Section 7.1.3).

For the details about the collection of pregnancy information, see Section 10.5.3.

8.4.6 Death events

Any death that occurs within the observation period will be reported as an SAE, if not covered by the exemptions to the SAE definition as defined in Sections 10.4.2, and 10.4.2.2, which do also apply for fatal cases. Date and cause of death will be recorded. If available, a copy of an autopsy report should be submitted if available upon request.

In case of a fatal event, the event term should not be “death” but the underlying event which led to death (death = outcome). If there is more than 1 AE in a fatal case, only for the AE leading to death the outcome “fatal” should be selected. If the cause of death is unknown and cannot be ascertained at the time of reporting, “unexplained death” should be documented as event term.

In addition to reporting as SAE, the death page of the CRF needs to be completed.

8.5 Genetics

Genetic analyses are planned in this trial if approved by the IRB/IECs and competent authorities. Blood (residual) and/or isolated peripheral blood mononuclear cells (PBMCs) will be taken at Visit 1 to evaluate the association between the human leukocyte antigen (HLA) and the immune response elicited by BNT165e or its components (BNT165c, BNT165d1, BNT165d2, referred to from here on as components). HLA typing data may complement additional analyses, e.g., characterization of T cell receptor repertoire and/or phenotypic characterization of antigen-specific T-cell responses using multimers. Data generated with these additional analyses may provide information about the HLA dependency of immune response (e.g., if distinct HLA types have stronger/better immune response after dosing with BNT165e or its components). Sampling for genetics (HLA typing and possibly RNA sequencing) will be performed at the time points listed in the SoA (Section 1.3). All data generated using the genetic samples will be handled in accordance with applicable laws and regulations; this includes requirements applicable for data protection, for any out-of-country sample shipment, and a potential withdrawal of consent.

8.6 Biomarker assessments

Biomarker analyses are planned in this trial if approved by the IRB/IECs and competent authorities. The biomarker/immunogenicity analyses comprise an analysis of biomarkers thought to play a role in the mechanism of action of BNT165e. Sampling for biomarkers/immunogenicity will be performed at the timepoints listed in Section 1.3.

Biological samples for biomarker/immunogenicity analysis may be retained for use for up to 15 years (or shorter if required by local regulations) after the end of the trial. The tube with the sample will be labeled with a number (optionally also with a bar code) to keep the subject's identity confidential; the tube label will not include information that could be used to directly identify the subject. Results of the blood analyses will be linked to the clinical information collected during the trial using this specific number. The analysis will only be carried out based on the label data and samples.

Biomarker/immunogenicity samples and all data generated using the samples will be handled in accordance with applicable laws and regulations. Leftover of biological samples (blood, the derived PBMCs, serum, or plasma) may be used for additional research analyses as described below. The leftover of biological samples may also be used for purposes to aid method development, assay qualification or validation and assay quality controls, biomarker studies related to BNT165e, and/or mechanisms of vaccine protection.

8.6.1 Immunogenicity assessments

Immunogenicity will be assessed at the timepoints listed in the SoA (Section 1.3).

Humoral immune response assessments will include:

- Characterization of the kinetics and magnitude of anti-CSP antibody responses.

Cell mediated immune (CMI) assessments will include:

- Evaluation of antigen-specific CD4+ and CD8+ T-cell responses measured by intracellular cytokine staining; this analysis will evaluate the frequency of CD4+ and CD8+ T cells expressing cytokines such as (but not limited to) interleukin -2 and interferon-gamma and possibly activation markers after *in vitro* stimulation with peptide pools from BNT165c, BNT165d1, and BNT165d2.

8.6.2 *Additional research analyses*

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8.7 Blood collection

The total blood volume drawn over any 8-week period in any group will always be less than 550 mL. Additional blood samples may be taken, e.g., for safety assessments after AEs or SAEs.

For the total volume of blood drawn for subjects in Part A see Section 1.3.

As long as the volume of blood drawn over any 8-week period is not increased, and as long as the maximum volume drawn per day does not exceed the cumulative blood volume specified for that visit in the SoA, the volume of individual blood draws may be adapted if required, e.g., due to unforeseen shortage of specific tubes.

Leftover of blood samples may be retained for up to 15 years and used for additional (exploratory) analyses, see Section 8.6. These leftover samples may also be used for future research involving the same IMP(s), the same or related therapeutic area, or for other relevant health research as well as unspecified (exploratory) research.

8.8 Unscheduled visits

In order to conduct evaluations or assessments required to protect the wellbeing of the trial subjects, the investigator may conduct unscheduled visits in addition to those listed in the respective SoA (Section 1.3).

8.9 Early termination visits

If, for any reason, subjects are permanently discontinued from the trial before completing all scheduled visits, subjects will complete an Early Termination Visit.

9 STATISTICAL CONSIDERATIONS

Methodology for summary and statistical analyses of the data collected in this trial is described here and further detailed in the statistical analysis plan (SAP), which will be maintained by the sponsor. The SAP may modify what is outlined in the protocol where appropriate; however, any major modifications of the primary endpoint definitions or their analyses will also be reflected in a protocol amendment.

There will be a trial IRC. For relevant procedures relating to IRC operation (e.g., charter, composition and schedule of meetings etc.), see Section 10.1.5.

9.1 Statistical hypotheses

No formal statistical hypotheses will be tested.

9.2 Interim analyses and analysis sequence

An interim analysis on safety and immunogenicity data will be performed when subjects in all cohorts of Part A have completed 28 d of follow-up after second injection. Also, interim analyses at other time points may be prepared to inform further clinical development.

The final analysis of Part A will be performed when the last trial subject enrolled in Part A completes the trial or is discontinued from the trial.

9.3 Sample size determination

In Part A, the sample size for each cohort is mainly driven by a dose-escalation trial in a limited number of subjects designed for early detection of potential safety and reactogenicity events, and is considered adequate to support the trial objectives, while minimizing the number of trial subjects exposed to a new IMP. The sample size of 10 subjects in Cohorts 1 to 6, 9 and 10 and of 15 in Cohorts 7 and 8 receiving the vaccine allow the detection of the most frequent AEs with high probability. With 10 subjects, the probability of detecting at least 1 AE is 65% if the underlying AE rate is 10%. With 15 subjects, the probability of detecting at least 1 AE is 79% if the underlying AE rate is 10%. Table 6 summarizes the probability of detecting at least one subject with an AE for AE rates of 10% and 15%, depending on the number of subjects.

Table 6: Probability of detecting at least one AE, depending on the sample size and event rate

Number of subjects	AE rate	Probability of detecting at least one subject with an AE
10	10%	65%
10	15%	80%
15	10%	79%
15	15%	91%

Abbreviation: AE = adverse event.

9.4 Analysis sets

The following analyses sets are defined:

Analysis Set	Description
Screened	All trial subjects who gave informed consent.
Safety Set	All trial subjects who received IMP (i.e., at least one dose of IMP).

Abbreviation: IMP = investigational medicinal product.

9.5 Statistical analyses

Statistical analyses will be performed by BioNTech or a designated CRO. All statistical analyses will be carried out using SAS®, Version 9.4 or higher, and/or other statistical software as required.

The SAP will be finalized before the first interim analysis, and it will include a more technical and detailed description of the statistical analyses described in this section. Any deviations from the planned analyses described in the final SAP will be described and justified in the clinical trial report. This section is a summary of the planned statistical analyses of the most important endpoints including the primary endpoints.

9.5.1 General considerations

In Part A, analyses will be performed by cohort for subjects receiving active IMP. Subjects who received placebo will be analyzed in a pooled manner across all cohorts.

Continuous variables will be summarized using the following descriptive statistics: number of trial subjects (n), mean and/or geometric mean depending on type of data, standard deviation, median, minimum, and maximum.

Categorical variables will be summarized presenting absolute and relative frequencies (n and %) of trial subjects in each category.

Baseline is defined as last available value before first dose of IMP.

9.5.2 Analysis of primary endpoints

The safety-based primary endpoints are defined in Section 3. Please refer to Section 9.5.2.1 for further details.

9.5.2.1 Safety endpoints

All safety analyses will be made on the Safety Set.

All AEs will be coded using the most recent version of Medical Dictionary for Regulatory Activities (MedDRA®) coding system.

Reactogenicity

Solicited local and systemic reactions (from the subject e-diary) will be summarized. In general, solicited reactions will be analyzed for each IMP injection, i.e.,

- Up to 7 d after each IMP injection

For each injection, the number and percentage of subjects reporting at least one local reaction or systemic reaction (i.e., solicited data collected using subject e-diaries) will be summarized for each of the following types:

- Any local reactions or systemic reactions
- Grade ≥3 local reactions or systemic reactions

Moreover, the number and percentage of subjects reporting at least one local reaction will be summarized by worst grade.

Unsolicited AEs

For Part A, the analysis of unsolicited AEs will include AEs that occur on the day of or after first IMP dose and up to 28 d after each dose, using the Safety Set. SAEs and MAAEs will be analyzed through 24 weeks after the last received dose of IMP.

The number and percentage of subjects reporting at least one AE (defined in Section 10.4.1) will be summarized by Preferred Term (PT) nested within system organ class for each of the following AE types:

- Any AE
- Related AE
- Related Grade ≥3 AE
- Any solicited local and systemic reaction that continues longer than 7 d post-vaccination or is an SAE or local reactions with an onset between Day 8 and Day 14 (included) after IMP administration
- Any SAE
- Related SAE
- Any MAAE
- Related MAAE

Moreover, the number and percentage of subjects with any AE will be summarized by worst grade by PT nested within system organ class.

Additional AE analyses may be described in the SAP.

9.5.3 Analysis of secondary endpoints

The secondary endpoints are defined in Section 3.

Secondary endpoints analyses will be described in the SAP.

9.5.4 Analysis of exploratory endpoints

The exploratory endpoints are defined in Section 3. Exploratory endpoints analyses will be described in the SAP, if relevant to the clinical trial report (CTR).

9.5.5 Analysis of other safety analyses

Other safety data that will be summarized includes clinical laboratory parameters, vital signs and ECGs. All safety analyses will be based on the Safety Set and will be summarized descriptively. The analysis of other safety data will be further described in theSAP.

9.5.6 Other analyses

Other analyses, if relevant to the CTR, will be described in the SAP.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, ethical, and trial oversight considerations

This trial will be conducted in accordance with this protocol, the ethical principles that have their origin in the Declaration of Helsinki, ICH GCP guidelines, and applicable laws and regulations.

10.1.1 Regulatory and ethical considerations

This trial will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH GCP Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, IB, and other relevant documents, will be submitted to the relevant regulatory authorities as required by applicable regulations. If required, approval for conducting the trial will be obtained from regulatory authorities in accordance with relevant regulatory requirements.

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) will be submitted to an IRB/IEC and reviewed and approved by the IRB/IEC before the trial is initiated. Advertisements may also be submitted after the trial is initiated.

Any amendments to the protocol will be submitted for IRB/IEC approval and (if required) competent authority approval before implementation of changes made to the trial design, except for changes necessary to eliminate an immediate hazard to trial subjects.

The principal investigator or delegate will be responsible for the following:

- Providing written summaries of the status of the trial to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.

- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
- Providing oversight of the conduct of the trial at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 (if applicable), and all other applicable local regulations.
- Informing the sponsor immediately about any urgent safety measures taken by the investigator to protect the trial subjects against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the investigator becomes aware of.

The principal investigator, any investigator(s), the sponsor, or personnel at other establishments, must cooperate with any inspection of the documents, facilities, records, and other resources deemed appropriate by the inspecting authorities to be related to the trial and that may be located at the trial site, at the sponsor, or at other establishments.

The sponsor must be notified as soon as possible about any upcoming regulatory authority inspection.

10.1.2 Financial disclosure

All investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the trial and for 1 year after completion of the trial.

10.1.3 Informed consent process

Informed consent must be obtained before any trial-specific screening procedure is performed.

Trial subjects must be informed that their participation is voluntary.

The investigator will explain the nature of the trial to the trial subject and answer all questions regarding the trial.

Trial subjects will be required to sign and date a statement of informed consent that meets the requirements of local regulations (e.g., 21 CFR 50), ICH GCP guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or trial site.

The medical record must include a statement that written informed consent was obtained before the trial subject was enrolled in the trial and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Trial subjects must be informed in a timely manner if new information becomes available that may impact their willingness to participate in the trial. If required, trial subjects will be re-consented to updated written information and consent forms.

Trial subjects who are rescreened must reconsent.

A copy of the ICF(s) must be provided to the trial subject.

Trial subjects must be re-consented to the most current version of the ICF during their participation in the trial.

10.1.4 Data protection

All data collected and processing during this trial will be performed in accordance with the applicable data protection requirements.

Trial subject personal data will be stored at the trial site in encrypted electronic and/or paper form and will be password protected or secured in a locked room to ensure that only authorized trial personnel have access. The trial site will implement appropriate technical and organizational measures to ensure that the personal data can be recovered in the event of disaster. In the event of a potential personal data breach, the trial site will be responsible for determining whether a personal data breach has in fact occurred and, if so, providing breach notifications as required by law.

Trial subjects will be assigned a unique identifier. The trial site will maintain a confidential list of trial subjects who participated in the trial, linking each trial subject's unique identifier to his or her actual identity and medical record identification.

Any trial subject records or datasets that are transferred to the sponsor will contain the identifier only; trial subject names or any information which would make the trial subject identifiable will not be transferred.

The trial subject must be informed that his/her personal trial-related data will be used by the sponsor in accordance with local data protection laws. The level of disclosure must also be explained to the trial subject who will be required to give consent for their data to be used as described in the informed consent.

The trial subject must be informed that their medical records may be examined by sponsor Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

If the trial subject withdraws from the trial and/or withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent. When trial subject data are to be deleted, the investigator will ensure that all copies of such data are promptly and irrevocably deleted from all systems.

10.1.5 Committees

10.1.5.1 Internal review committee (IRC)

A trial IRC will be established to provide medical oversight of trial subject safety, reactogenicity, and immunogenicity during the conduct of this trial, with a focus on guidance, management of emergent safety issues, and recommendations for dose-escalation as outlined in the IRC Charter. This includes periodic in-depth review of safety data by trial subject, cohort, and cumulatively, in order to confirm mechanism of action, identify potential off-target toxicities, assessment of any events of myocarditis or pericarditis for the level of diagnostic certainty as per established standard definition (Brighton Collaboration Case Definition) and to understand the IMP's safety and immunogenicity profile and feasibility for further clinical development. The IRC is also a forum for the discussion of other data which could impact the IMP benefit-risk assessment, thereby allowing the IRC to periodically assess the overall benefit-risk of the IMP.

The IRC will be constituted and act according to procedures described in the IRC Charter. The IRC will prepare written minutes of its meetings. The IRC may escalate potential findings to the IDMC.

All SAEs considered related to IMP either by the investigator or the sponsor will trigger an *ad hoc* review by the IRC.

10.1.5.2 Independent Data monitoring committee (IDMC)

The IDMC will be responsible for ongoing monitoring of the safety of subjects in this trial, according to the charter. This may include but is not limited to a quarterly review of cumulative safety data of all clinical trial participants. SUSARs and possible cases of myocarditis, pericarditis or myopericarditis will trigger an *ad hoc* review by the IDMC. Possible cases of myocarditis, pericarditis, or myopericarditis will be independently assessed by the IDMC using the Brighton Collaboration Case Definition.

The recommendations made by the IDMC will be forwarded to the IRC for review and final decision.

10.1.6 Dissemination of clinical trial data

A final ICH E3 conform report integrating primary and secondary, as well as selected exploratory endpoints results will be prepared by the sponsor.

In all cases, trial results will be reported in an objective, accurate, balanced, and complete manner and are reported regardless of the outcome of the trial or the country in which the trial was conducted.

Clinical trial data and documentation will be disseminated as required per applicable laws and regulations, e.g., the European Union (EU) Regulation No 536/2014, EU Regulation 1049/2001, and the US Final Rule, which implements Section 801 of the Food and Drug Administration Amendments Act (FDAAA 801). Clinical documents under such laws includes protocols and protocol amendments, SAPs, ICH E3 clinical study reports.

This trial will be registered, and trial results be publicly posted, on publicly accessible trial registries (e.g., ClinicalTrials.gov, EU Clinical Trials Register, etc.) as required per applicable laws and regulations.

If this clinical trial is used to support marketing authorization packages/submissions, the sponsor will comply with the EU Policy 0070, the proactive publication of clinical data on the EMA website. Clinical data, under Phase 1 of this policy, includes clinical overviews, clinical summaries, ICH E3 clinical study reports, and appendices containing the protocol and protocol amendments, sample CRFs, and statistical methods. Under Phase 2 of this policy, “clinical data” includes the publishing of individual trial subject data.

Even if not required by applicable laws and regulations, this trial will be registered, and trial results be publicly posted on ClinicalTrials.gov. In addition, expert summaries of the outcomes for all primary and secondary outcome measures (irrespective of outcome) and lay summaries, will be posted on a publicly accessible website.

The results for all primary and secondary outcome measures, irrespective of outcome, will be submitted for publication in academic journals (for further details, see Section 10.1.10).

10.1.7 Data quality assurance

All trial subject data relating to the trial will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must permit trial-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities and requirements, including handling of non-compliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

The sponsor or designee is responsible for the data management of this trial including quality checking of the data.

The sponsor assumes accountability for actions delegated to other parties (e.g., CRO).

Ongoing source data verification will be performed to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of trial subjects are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICFs, pertaining to the conduct of this trial must be retained by the investigator for 15 years after trial completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8 Source documents

Source documents provide evidence for the existence of the trial subject and substantiate the integrity of the data collected. Source documents are filed at the investigator’s site.

Data reported on the CRF or entered in the CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the trial. Also, current medical records must be available.

Source data are all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source documents are original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, trial subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

10.1.9 Early trial site closure and trial termination

A trial site is considered closed when all required documents and trial supplies have been collected and a trial site closure visit has been performed.

All trial sites will be closed upon trial completion.

The investigator may initiate trial site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended trial site closure.

The sponsor reserves the right to close a trial site early or to terminate the whole trial or to suspend the whole trial (a temporary halt; an unplanned interruption of the conduct of a trial by the sponsor with the intention to resume it) at any time for any reason.

Reasons for the sponsor to close a trial site early include (but are not limited to):

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines;
- Inadequate recruitment of trial subjects by the investigator.

Reasons for the sponsor to prematurely terminate the whole trial include (but are not limited to):

- When there are safety concerns (e.g., if there is an IRC recommendation);
- Discontinuation of further trial treatment development.

If the trial is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any CROs used in the trial of the reason, as specified by the applicable regulatory requirements.

10.1.9.1 Investigator tasks triggered by premature termination of the trial

If the trial is prematurely terminated for any reason, the investigator should promptly inform the trial subjects, should assure appropriate therapy and follow-up for the subjects, and, where required by the applicable regulatory requirement(s), should inform the regulatory authorities. In addition:

- If the investigator terminates a trial without prior agreement of the sponsor, the investigator should inform the institution where applicable, and the investigator should promptly inform the sponsor and the IRB/IEC, and should provide the sponsor and the IRB/IEC a detailed written explanation of the termination.
- If the sponsor terminates a trial, the investigator should promptly inform the institution where applicable and the investigator should promptly inform the IRB/IEC and provide the IRB/IEC a detailed written explanation of the termination.
- If the IRB/IEC terminates its approval/favorable opinion of a trial, the investigator should inform the institution where applicable and the investigator should promptly notify the sponsor and provide the sponsor with a detailed written explanation of the termination.

10.1.10 Publication policy

The results for all primary and secondary outcome measures, irrespective of outcome, will be submitted by the sponsor for publication in academic journals. The results of this trial may also be presented by the sponsor at scientific meetings.

The results of this trial may be published or presented at scientific meetings by the investigator. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments. The investigator will, on request, remove any previously undisclosed confidential information before disclosure, except for any trial treatment-related information necessary for the appropriate scientific presentation or understanding of the trial results.

Unless agreed in advance otherwise, site- or subpopulation-specific analyses may only be published after the outcomes of the primary endpoint analyses have been published.

The sponsor will comply with applicable requirements for publication of trial results. In accordance with standard editorial and ethical practice, including those established by the International Committee of Medical Journal Editors (ICMJE). The sponsor will generally support publication of multi-site trials only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with ICMJE authorship requirements.

10.1.11 Protocol preparation and approval

This protocol has been prepared, reviewed and approved in accordance with the sponsor's SOP. Documentation of this process is filed in the Trial Master File (TMF).

10.1.12 Investigators and trial administrative structure

10.1.12.1 Investigators and trial site personnel

There must be an investigator at each trial site.

If the trial is conducted by a team of individuals at the trial site, the investigator leading and responsible for the team is called the principal investigator.

The responsibilities of principal investigator(s) must be documented before any trial-related procedure is performed. All persons assigned responsibility as principal investigator must sign a declaration of their responsibilities. They must also sign a declaration that they have read and understand the content of the protocol, that all questions have been adequately answered, and that they are qualified by experience and training to act as investigator for this trial.

The principal investigator at each trial site is responsible for ensuring that this trial is conducted in accordance with the protocol, the principles of GCP, and applicable regulatory requirements.

If the trial is conducted at multiple trial sites, a coordinating investigator must be assigned who is responsible for the coordination of investigators at different trial sites. The responsibilities of the coordinating investigator must be documented before any trial-related procedure is performed.

Documentation of all involved investigators must be maintained according to ICH GCP and applicable regulatory requirements.

Curriculum vitae and/or other relevant documents confirming the current qualification of the investigators must be provided to the sponsor. This should include any previous

training in the principles of GCP, experience obtained from work with clinical trials, and experience with medical care.

10.1.12.2 Trial site personnel assigned trial-related duties

The principal investigator may define appropriately qualified personnel at a trial site to perform significant trial-related procedures and/or to make trial-related decisions under his/her supervision. In this case, the principal investigator must maintain a signed list of the persons to whom they delegate significant trial-related duties/responsibilities; the delegated trial-related duties/responsibilities must be specified in the list.

When personnel or responsibility changes are made, the principal investigator must ensure that the relevant documentation is updated before any trial-related activities are performed.

Documentation of all involved trial site personnel performing significant trial-related procedures and/or making trial-related decisions must be maintained according to GCP and applicable regulatory requirements.

10.1.12.3 Contract research organizations

Documentation of all involved CRO must be maintained according to GCP and applicable regulatory requirements. This includes documentation of any delegation of responsibilities to CROs.

10.1.12.4 The sponsor and sponsor's personnel

The trial sponsor listed on the title page accepts the responsibilities of the sponsor according to GCP and applicable regulatory requirements.

The sponsor will designate appropriately qualified personnel to advise on trial-related topics. The trial site will be provided with contact details for these personnel before any trial-related procedure is performed.

A list of key sponsor personnel involved in the preparation of this protocol and the conduct of the trial, including their full names, titles, roles, and responsibilities, will be maintained.

10.2 Data collection and management

The trial documentation must be adequate for the reconstruction of the trial.

10.2.1 Data management

The CRO will be responsible for data management of this trial, including quality checking of the data.

Data entered manually will be submitted to the sponsor through use of an EDC system, data extracts, and reports. Trial sites will be responsible for timely data entry into the EDC system as defined in the CRF completion guidelines. In the event of discrepant data, the CRO will request data clarification from the trial sites, which the trial sites will resolve electronically in the EDC system.

The CRO will produce a Trial Data Validation Specification document that describes the quality checking to be performed on the data. CRFs and correction documentation will be maintained in the EDC system's audit trail.

Centrally evaluated clinical laboratory tests and ECG data will be sent directly to the data management service provider.

System backups for data stored by the sponsor and records retention for the trial data will be in accordance with regulatory requirements.

At the end of the trial, the investigator will receive trial subject data for his/her trial site in a readable format that must be kept with the trial records. Acknowledgment of receipt of the trial subject data will be obtained.

10.2.2 Case report forms

CRFs will be completed through use of an EDC system. Trial site personnel will receive training and have access to a manual for appropriate CRF completion. The CRFs should be handled in accordance with instructions and be submitted electronically to the sponsor via the system.

All CRFs should be completed in a timely manner by designated, trained trial site personnel. CRFs should be reviewed, verified, and then electronically signed and dated by the investigator or a designee.

10.2.3 Subject reported outcomes

Subject e-diaries will be used for the reporting of reactogenicity (local reactions and systemic events) by the trial subjects. See Section 8.3.6 for details).

Data entered into the e-diary is transmitted to EDC via a subject cloud mobile gateway. During the whole process (while data is at rest on the device and during transmission) the data is encrypted in the highest level that is legally allowed. After successful transmission the data is no longer held on subjects' device.

10.2.4 Investigator's Site File and the Trial Master File

The principal investigator is responsible for the filing of all essential documents in an ISF. The sponsor is responsible for the timely filing of all essential documents in the TMF. As applicable, these files must be available at monitoring visits and during audits or regulatory inspections.

After trial completion, the principal investigator must ensure that all source data/documentation related to the trial is recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification. The principal investigator must take measures to prevent accidental or premature destruction of these documents.

The principal investigator must keep the ISF, the source data/documentation arising from the trial according to the prescribed record retention period in the country and/or according to the hospital policy, but at least until informed by the sponsor that the trial-related records are no longer required.

10.3 Clinical laboratory tests

See Section 8.3.4 for details about the clinical laboratory tests planned in this trial. There is no additional information.

10.4 Adverse events: Definitions and procedures for recording, evaluating, follow-up, and reporting

10.4.1 Definition of AE

AE definition:

- An AE is any untoward medical occurrence in a trial subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

- An MAAE is an AE for which the subjects received medical attention defined as hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits.

10.4.1.1 Events <u>meeting</u> the AE definition:

- Any abnormal clinical laboratory test results (e.g., clinical chemistry results) or other safety assessments (e.g., ECG, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or severity of the condition.
- Serious AEs occurring after the date informed consent was given (ICF signed and dated) and AEs (non-serious AEs) occurring after first IMP administration.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either trial treatment or a concomitant medication. Overdose *per se* will not be reported as an AE/SAE.

10.4.1.2 Events <u>not meeting</u> the AE definition:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments, unless judged by the investigator to be more severe than expected for the trial subject’s condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur or continue (social and/or convenience admission to a hospital).

10.4.1.3 Documentation of particular situations

10.4.1.3.1 AEs that are secondary to other events

In general, AEs that are secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. A medically significant secondary AE that is separated in time from the initiating event should be documented as an independent AE in source data and CRF. For example:

If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be documented as AE.

If vomiting results in severe dehydration, both events should be documented as AEs separately.

10.4.1.3.2 Abnormal laboratory results and vital sign values

Not every laboratory or vital signs abnormality needs to be documented as AE. For clinically significant laboratory/vital signs abnormalities the following definitions and documentation rules apply:

- If a laboratory/vital signs abnormality is a sign of a disease or syndrome, the laboratory/vital signs abnormality is clinically significant and only the diagnosis of the causing disease or syndrome needs to be documented as AE.
- If a laboratory/vital signs abnormality results in specific symptoms but no diagnosis of a disease or syndrome can be made, the laboratory/vital signs abnormality is clinically significant and only the symptoms need to be documented as AEs.
- If a laboratory/vital signs abnormality is not a sign of a disease or syndrome and does not result in specific symptoms but leads to a change in trial treatment or in a medical intervention, the laboratory/vital signs abnormality is clinically significant and must be documented as AE.

10.4.1.3.3 AEs associated with an overdose or error in drug administration

An overdose is the accidental or intentional use of a drug in an amount (per administration or cumulatively) higher than the dose being studied (for the trial treatment) or higher than the maximum recommended dose according to the authorized product information (for approved concomitant medications). An overdose or incorrect administration of a drug is not itself an AE, but it may result in an AE.

All AEs associated with an overdose or incorrect administration should be documented as AE in source data and CRF and reported as SAE if applicable.

10.4.1.4 Suspected adverse reaction

Suspected adverse reactions are untoward and unintended responses to an IMP related to any dose administered.

The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

The definition implies a reasonable possibility of a causal relationship between the event and the IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship.

10.4.2 Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met.

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death
- Is life-threatening

  The term “life-threatening” in the definition of “serious” refers to an event in which the trial subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
- Requires hospitalization or prolongation of existing hospitalization:
  - In general, hospitalization signifies that the trial subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether “hospitalization” occurred or was necessary, the AE should be considered serious.
  - Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in persistent disability/incapacity:
  - The term disability means a substantial disruption of a person’s ability to conduct normal life functions.
  - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly or birth defect.
- Other situations:
  - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the trial subject or may require medical or surgical treatment to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
  - Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.4.2.1 Use of the terms “severe” and “serious”

The terms “severe” and “serious” are not synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe, or potentially life-threatening, as described in the US FDA 2007); see Section 10.4.3 for guidance on the assessment of severity; the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be assessed independently for each AE recorded on the CRF.

SAEs must be reported by the investigator to the sponsor within 24 hours after learning of the event; see Section 10.4.4 for reporting instructions.

10.4.2.2 SAE exemptions

In general, SAEs are defined according to ICH Topic E2A (CPMP/ICH/377/95), EU Directive 2001/20/EC and ENTR/CT-3 (see Section 10.4.2). In the present trial, some events are excluded from the SAE definition. The following events do not need to be reported as SAEs:

- AEs and SAEs occurring after the end of the observation period must only be reported by the investigator to the sponsor if a relationship to trial treatment or trial procedure is suspected.
- Hospitalizations for respite care will not be considered as reportable SAE.
- Hospitalizations solely for coordination of care, including hospice arrangements, will not be considered as reportable SAE.
- Hospitalizations that were necessary solely because of trial subject requirement for care outside of normal outpatient clinic operating hours will not be considered as reportable SAE.
- Planned hospitalizations required by the protocol (e.g., for trial treatment administration or insertion of access device for trial treatment administration) will not be considered as reportable SAE.
- Hospitalizations for procedures or interventions of a pre-existing condition of the trial subject (elective surgery = planned, non-emergency surgical procedure) will not be considered as a reportable SAE (unless the intervention/procedure is not caused by an acute worsening of the pre-existing condition during the time trial participation):
- If it was planned and documented in trial subject record before the trial-specific trial subject informed consent was signed (ICF for trial participation, see Section 10.1.3), or
- If it was scheduled during the trial when elective surgery became necessary, and the trial subject has not experienced an AE.

Note: nevertheless, this kind of hospitalization should be avoided during trial treatment.

10.4.3 Recording and follow-up of AEs and/or SAEs

AE and SAE recording

The investigator needs to assess and document any AE regardless of association with the use of the trial treatment during the period of observation (see Section 8.4.1).

- Data pertaining to AEs will be collected during each trial visit either. Based on the trial subject's spontaneous description or investigator's inquiry or discovered in the course of examinations done during the visit, clinical significance of any sign or symptom needs to be evaluated by the investigator.
- Clinically significant findings need to be documented as AEs in the source data and CRF. Findings that are evaluated and documented in the source data as not clinically significant (e.g., an abnormal laboratory value without any clinical manifestation), should not be documented as AE.
- The investigator will then record all relevant AE information in the CRF and perform an assessment on:
  - Severity (see the section Assessment of severity)
  - Seriousness
  - Outcome including onset and end dates
  - Causal relationship of the AE to the trial treatment/trial procedure
  - Any trial treatment action and/or any other action taken
- All assessments as well as AE term (diagnosis/description), start date and time of onset, end date and time need to be documented in the CRF.
- There may be instances when copies of medical records for certain cases are requested by the sponsor. In this case, all trial subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to the sponsor.
- To avoid colloquial expressions, the AE should be reported in standard medical terminology. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE. If a definitive diagnosis is not possible, the individual signs and symptoms should be recorded.

Assessment of severity

The assessment of AE and/or SAE intensity should be done consistently for all trial subjects treated with the same treatment and dose. In case of doubt, the medical monitor should be consulted.

The intensity of (serious) AEs will be graded by the investigator. For further guidance refer to the FDA Guidance for Industry (US FDA 2007). Where specific guidance for an adverse event term is not provided, the following general approach should be followed:

- Grade 1 – Mild; does not interfere with the trial subject's usual function.
- Grade 2 – Moderate; interferes to some extend with the trial subject's usual function.

- Grade 3 – Severe; interferes significantly with the trial subject's usual function.
- Grade 4 – Potentially life-threatening; life-threatening consequences, urgent intervention required.

With regards the intensity of an (serious) AE, the following needs to be documented in the CRF:

- Initial intensity of the AE
- For each change of intensity:
- New grade of intensity
- Date of change (= start of new grade of intensity)
- Time of change (only if relevant)
- New action taken

A change of severity only needs to be documented if there is a clearly definable change in grading of the AE (e.g., a laboratory result changes from severe to moderate).

An event is defined as "serious" when it meets at least one of the predefined seriousness criteria as described in the definition of an SAE, independent from its intensity.

Actions taken by the investigator

Actions taken by the investigator as a result of an AE must be documented.

Action(s) taken with trial treatment (IMPs) by the investigator:

- Dose not changed (= continuation of trial treatment administration according to the trial protocol)
- Drug interrupted (i.e., trial treatment withdrawn temporarily, interruption and resumption);
    - Cancellation of IMP administration at a given visit
    - Interruption of IMP administration during a given visit
- Drug withdrawn (i.e., trial treatment permanently withdrawn)
- Unknown (e.g., in case the trial subject is lost to follow-up)
- Not applicable (e.g., in case treatment with trial treatment has not yet started or event starts after last trial treatment administration)

Other action(s) that may be taken by the investigator include:

- None
- Initiation of a remedial (concomitant) therapy
- Other specific treatment(s) of AE (to be specified)

Investigator assessment of the outcome of an AE

The investigator has to assess the outcome of an AE (and not the trial subject's outcome) at the time of documentation based on the following criteria:

- Recovered/resolved* (= complete resolution of the AE)
- Recovering/resolving (= AEs which are improving but not yet resolved completely, e.g., decreases in severity grade)
- Not recovered/not resolved (= AEs which are ongoing without improving or still present when the trial subject deceases due to another cause)
- Recovered/resolved with sequelae* (= trial subject recuperated but retained pathological conditions resulting from the AE; the sequelae should be indicated)
- Fatal** (= death due to the AE)
- Unknown (e.g., in case the trial subject is lost to follow-up)

* Generally, an AE is defined as recovered/resolved if all symptoms have ceased, no medication for treatment of the event is taken anymore and no other measures (e.g., hospitalization) are ongoing.

If the trial subject has developed permanent or chronic symptoms or if the event requires long-term medication(s), the AE is defined as recovered/resolved with sequelae as soon as no changes of symptoms and/or medication(s) are expected anymore.

An AE that is documented as a worsening of a medical condition already known at baseline, is defined as recovered as soon as the medical condition has returned to baseline status.

** In case of a fatal event, the event term should not be “death” but the underlying event which led to death (death = outcome). If there is more than one AE in a fatal case, only the AE leading to death will be attributed with the outcome “fatal”. All other AEs ongoing at the time of death will be attributed with the outcome “not recovered/not resolved”. A copy of an autopsy report should be submitted if available.

Assessment of causality

The investigator is obligated to assess the relationship between each occurrence of each SAE and trial treatment and/or trial procedure.

The investigator will use clinical judgment to determine the relationship.

Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to trial treatment administration will be considered and investigated.

It is sufficient to document the causality in the source data and CRF as:

- Related (= there is a reasonable possibility of a causal relationship) or
- Not related (= there is no reasonable possibility of a causal relationship)

The relationship or association of an AE or SAE to a trial treatment/trial procedure will be assessed by the investigator after having evaluated all accessible data and, if necessary, the investigator will re-evaluate the case as new information becomes available.

Events caused by the procedure of trial treatment administration should be differentiated from events caused by the trial treatment itself. Only events suspected to be caused by the

IMPs itself should be documented as suspected adverse reactions but not events caused by the procedure of trial treatment administration.

In this trial, it cannot be excluded that during the course of the trial some procedures give rise to AEs which are related to the trial procedure and not to the trial treatment. Procedure-related AEs can occur during or after trial-specific procedures (e.g., blood draws, injections). These events must be reported in the CRF on Adverse Event pages as "related to trial procedure" with the causing procedure specified.

Notes

There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to the sponsor. However, it is very important that the investigator always makes an assessment of causality for every event before the initial transmission of the SAE data to the sponsor.

The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

The causality assessment is one of the criteria used when determining regulatory reporting requirements.

10.4.4 Reporting of SAEs

For the period of observation please refer to Section 8.4.1.

All SAEs which occur in a trial subject during the observation period, whether considered to be associated with trial medication or not, must be reported by the investigator to the sponsor within 24 h following first knowledge of the event.

All SAEs occurring after the end of the period of observation only have to be reported to the sponsor if the investigator suspects a relationship to trial medication or the trial procedure.

SAE Reporting to the sponsor using paper report forms

The investigator must ensure the respective paper report form is completed and transmitted to the sponsor via one of the following reporting lines:

- Safety Report Fax No.: [redacted]
- Safety Report E-Mail address: [redacted]

In parallel the respective SAE should be recorded in EDC as soon as possible.

Information for the final description and evaluation of a case report may not be available within the required time frames for reporting. Nevertheless, for regulatory purposes, initial reports should be submitted if the following minimal information is available:

- An identifiable trial subject (subject number)
- A suspected medicinal product
- An identifiable reporting source (investigator/trial site identification)
- An event or outcome that can be identified as serious

For SAEs, follow-up information should be sent to the sponsor (indicating that this is a "follow-up" report using the respective SAE Form) without delay as described above and

accompanied by appropriate anonymous supporting documentation (e.g., discharge letters, medical reports or death certificates), until a final outcome and date are available. All confidential information (name, address, full day of birth) needs to be blackened before sending.

A copy of the submitted SAE report must be retained on file by the investigator. If explicitly required according to national legislation, the investigator must submit copies of the SAEs to the IRB/IEC or authority and retain documentation of these submissions in the Site Trial File.

In case an investigator or any other trial team member has questions on safety reporting the sponsor may be contacted via:

- E-Mail: [redacted]

For medical questions, the sponsors medical monitor for this trial should be contacted.

10.5 Contraceptive guidance and collection of pregnancy information

The following definitions and guidance are based on the Clinical Trial Facilitation Group (CTFG) recommendations related to contraception and pregnancy testing in clinical trials issued in 2020 (CTFG 2020).

10.5.1 Definitions

Volunteers of childbearing potential

For the purpose of this document, a volunteer born female is considered of childbearing potential, i.e., fertile, following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

If fertility is unclear (e.g., amenorrhea in adolescents or athletes or during cancer treatment), additional evaluation should be considered.

Volunteers born female in the following categories are not considered VOCBP:

- Premenarchal
- Premenopausal female with one of the following:
- Reported hysterectomy
- Reported bilateral salpingectomy
- Reported bilateral oophorectomy

For trial subjects with permanent infertility due to a medical cause other than the above, (e.g., mullerian agenesis, androgen insensitivity), investigator discretion should be applied to determining trial entry.

Postmenopausal state

For the purpose of this document, a postmenopausal state is defined as no menses for 12 months without an alternative medical cause (verified by FSH levels). A high FSH level

in the postmenopausal range will be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT).

Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the trial. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before trial enrollment.

Fertile men

For the purpose of this document, a man is considered fertile after puberty unless permanently sterile by bilateral vasectomy or orchidectomy.

10.5.2 Contraception guidance

The following guidance describes what is considered highly effective and acceptable methods of contraception.

The investigator or delegate should advise the trial subject how to achieve highly effective contraception.

The following birth control methods that can achieve a failure rate of less than 1% per year when used consistently and correctly may be considered as highly effective:

- Combined estrogen and progestogen-based hormonal contraception associated with inhibition of ovulation [1], i.e., established use of (oral, intravaginal, or transdermal) hormonal methods of contraception.
- Progesterone-only based contraception associated with inhibition of ovulation [1], i.e., established use of (oral, injected, or implanted) hormonal methods of contraception. [2]
- Intrauterine device. [2]
- Intrauterine hormone-releasing system. [2]
- Bilateral tubal occlusion. [2]
- Bilateral vasectomy (for a male trial subject or male partner of a female trial subject). [2, 3]
- Sexual abstinence. [4]

1. Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method.
2. Contraception methods that in the context of this guidance are considered to have low user dependency.
3. A vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the VOCBP trial subject and that the vasectomized partner has received medical assessment of the surgical success.
4. In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the trial subject.

10.5.3 Collection of pregnancy information

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication (spontaneous abortion, bleeding, preeclampsia, etc.) or elective termination of a pregnancy will be reported as an AE or SAE.

A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-trial pregnancy-related SAE considered reasonably related to the trial treatment by the investigator will be reported to the sponsor. While the investigator is not obligated to actively seek this information in former trial subjects, the investigator may learn of an SAE through spontaneous reporting.

Pregnancy information will only be collected after obtaining written informed consent from the pregnant trial subject (or if a male trial subject's partner becomes pregnant, written informed consent from the partner or from both depending on the national regulations).

The initial and follow-up information must be documented on the paper-based Pregnancy Reporting Form and submitted to the sponsor within 24 h of learning of a trial subject's pregnancy/partner's pregnancy and in the CRF. The completed form needs to be sent to the Safety Report Fax number or E-Mail given in Section 10.4.4. Completed pregnancy forms must be signed by an investigator before faxing/mailing them to the sponsor. Blank reporting forms are provided to the investigator during the site initiation visit and are filed in the ISF.

The investigator will collect follow-up information on the trial subject/trial subject's partner and the neonate, and the information will be forwarded to the sponsor. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth. In order to collect further follow-up information on the health status of the newborn and its development, both parents need to provide written consent. Information to be collected includes the presence or absence of any congenital abnormalities, birth defects, maternal or newborn complications and their presumed relation to the IMP. Generally, the follow-up will be of a duration determined in consultation with the pediatrician.

10.5.4 Management of contraception

At time points indicated in the SoA (Section 1.3), the investigator or designee will inform the trial subject of the need to use the prescribed contraception consistently and correctly, and will counsel the subject not to donate or cryopreserve germ cells In addition, the investigator or designee will instruct the trial subject to call immediately if the selected contraception method is discontinued or if pregnancy is known or suspected in the trial subject or partner.

The investigator or designee, in consultation with the trial subject, will confirm that the trial subject has selected an appropriate method of contraception for the individual trial subject and his or her partner(s) from the permitted list of contraception methods and will confirm that the trial subject has been instructed in its consistent and correct use.

VOCBP will only be administered trial treatment after negative pregnancy test outcomes at the time points indicated in the SoA (Section 1.3).

10.6 Liver safety: Suggested actions and follow-up assessments

Not applicable.

10.7 Standard definitions

10.7.1 Definition of screened subject

After providing informed consent, volunteers are considered screened in this trial, i.e., are trial subjects.

10.7.2 Definition of solicited and unsolicited safety data

All reactogenicity events information recorded via the subject e-diaries are solicited events.

10.7.3 Definition of trial completer

A trial subject is considered to have completed the trial if they have completed all phases of the trial including the last visit or the last scheduled procedure shown in the SoA (see Section 1.3).

10.8 Country-specific requirements

Not applicable.

10.9 Prior protocol amendments and version updates

The protocol amendment summary of changes for the current update is located directly before the table of contents. A comparison of every new sponsor approved protocol version with the next approved version is filed together with the protocol in the TMF.

10.9.1 Protocol update from version 1.0 to 2.0

This amendment implements changes in the primary endpoint, SoA, trial design, trial treatment, inclusion and exclusion criteria, pausing rules, and treatment for specific adverse reactions CCI [redacted]. Other changes were clarifications (see the below tabular summary).

Detailed description of changes

Minor editorial changes, such as rewording or improvement of language for consistency, are not specifically listed.

See the table for a summary of the reasons for changes compared to the previous version:

Section	Reason for change
All sections	Omission of Part B from the protocol CCI [redacted].
1.1, 1.3, 3 and 8.4.1	Change in the MAAEs collection time.
5.2	Correction in the QTcF used to define an abnormal ECG reading.
6.1	Reference to the “Pharmacy Manual” was added.
6.10.1	4-week timeframe was deleted so that the subjects with symptoms potentially caused by myocarditis or pericarditis will be evaluated for the duration of the study, CCI [redacted].
6.6.1	Correction on the randomization text for Part A.
7.1.1	Changes in pausing rules CCI [redacted].
8	The sentence referring to protocol waivers and exemptions was updated: “Protocol waivers or exemptions are not allowed”.

Section	Reason for change
8.3.4	Addition of "creatinine" in the clinical laboratory parameters.
8.2.6	Clarification on subject's e-diary CCI [redacted].

10.9.2 Protocol update from version 2.0 to 3.0

This amendment implements changes in the follow-up duration of AEs, MAAEs and SAEs, pausing rules and assessment of local and systemic reactions CCI [redacted]. Other changes were clarifications (see the below tabular summary).

Detailed description of changes

Minor editorial changes, such as rewording or improvement of language for consistency, are not specifically listed.

See the table for a summary of the reasons for changes compared to the previous version:

Section	Reason for change
Section 5.5	Removal of wordings "for multiple or individual cohorts" to not restrict pausing rules to a specific cohort.
Section 7.1.1	Modification in the pausing rules by removing "in a given cohort" to not restrict the subjects' safety for a particular cohort CCI [redacted].
Section 8.3.6	Clarification on the wordings of local and systemic reactions assessment especially Grade 3 and 4 and aligned it with e-diaries CCI [redacted].
Section 8.4.1	Changes in the AEs, SAEs and MAAEs follow-up time CCI [redacted].

10.9.3 Protocol update from version 3.0 to 4.0

The amendment (updating from version 3.0 to 4.0) implements changes in the sentinel dosing approach, the criteria for permanent discontinuation of single trial subjects from trial treatment, and clarifies the future use of stored samples CCI [redacted]. Other changes were clarifications (see the below tabular summary).

Detailed description of changes

Minor editorial changes, such as rewording or improvement of language for consistency, are not specifically listed.

Section	Reason for change
Title page	Changes reflecting this update and the addition of now available regulatory identifiers.
Page 2	This section was updated to document this update.
Section 1.1	The trial title was aligned with the one given on the title page. Figure 1 was updated to reflect CCI [redacted] an increased number of sentinel subjects. The text was updated to reflect CCI [redacted] an increased number of sentinel subjects. An inconsistency between the synopsis and the Section 7.1.1 text related to pausing rules was corrected. Synopsis subsections mistakenly included in Section 1.2 (Schema) were moved to Section 1.1 (Synopsis).

Section	Reason for change
Section 1.2	Synopsis subsections mistakenly included in Section 1.2 were moved to the correction location, Section 1 (Synopsis).
Section 4.2	The text was updated to reflect CCI [redacted] an increased number of sentinel subjects.
Section 7.1.3	The criteria for permanent discontinuation of single trial subjects from vaccination was updated CCI [redacted].
Section 8.6	Clarification text regarding the use of leftover samples was added CCI [redacted].
Section 10.2.3	Clarification text regarding the data flow for e-diary data was added CCI [redacted].
Section 10.4.3	Text in the subsection “Assessment of causality” was updated for clarification.
Section 10.5.1	Text in the subsection “Definitions” was updated for clarification.
Section 10.9.3	This section was added to document this update.

10.9.4 Protocol update from version 4.0 to 5.0

The amendment (updating from version 4.0 to 5.0) implements an addition of cohort and thus the sample size was increased to more fully investigate the immunogenicity dose-response curve of BNT165c. Additionally, to improve operational flexibility, a few visit windows were broadened slightly. Other changes were clarifications (see the below tabular summary).

Detailed description of changes

Minor editorial changes, such as rewording or improvement of language for consistency, are not specifically listed.

Section	Reason for change
Section 1.2	Addition of Cohort 9 (≤100 µg BNT165c). Changes implemented to the sample size and schema accordingly.
Section 1.3	Some of the visit windows are updated in the SoA.
Section 9.3	Addition of Cohort 9.

10.9.5 Protocol update from version 5.0 to 6.0

The amendment (updating from version 5.0 to 6.0) implements the addition of a new cohort to evaluate a different dosing schedule that may be implemented in efficacy trial(s), so the sample size of the trial was increased. Other changes were clarifications (see the below tabular summary).

Detailed description of changes

Minor editorial changes, such as rewording or improvement of language for consistency, are not specifically listed.

Section	Reason for change
Sections 1.1 and 1.2	Addition of Cohort 10 (≤70 µg BNT165c and ≤30 µg BNT165d). Changes implemented to the sample size and schema accordingly. Addition of new schedule with IMP dosing Day 1, Day 29 and Day 57 for Cohort 10.

Section	Reason for change
Section 1.3	Addition of a new SoA for Cohort 10 that includes IMP dosing at Days 1, Day 29 and Day 57.
Section 2.2.2	Deletion of second bullet point i.e., Production.
Section 4	Section has been updated to reflect same changes as done in Section 1.1.
Sections 5 and 6	Visit numbers are deleted and replaced with just the days post dose since visit numbering is different for Cohort 10.
Section 7.1.1	The language of one of the cohort trial pausing rules is clarified.
Section 9.3	Addition of Cohort 10.

10.9.6 Protocol update from version 6.0 to 7.0

The amendment (updating from version 6.0 to 7.0) adds information on dosing in case of a trial pause in Part A.

Other changes were clarifications (see the below tabular summary).

Detailed description of changes

Minor editorial changes, such as rewording or improvement of language for consistency, are not specifically listed.

Section	Reason for change
1.1	Deletion of RTS,S related text to avoid duplication with Section 2 and addition of R21/Matrix-M (another World Health Organization recommended vaccine).
1.1, 4.1 and 4.1.2	Clarification on number of subjects due to trial pause and replacement of discontinued subjects.
1.1 and 4.1.1	Clarification on trial duration due to trial pause.
1.3	Clarification on assessment in early terminated subjects, visit windows and collection of adverse events (AEs), medically attended AEs (MAAEs) and serious AEs (SAEs) due to trial pause in Table 2 and Table 3
2.1	Addition of R21/Matrix-M information with relevant references.
5.2	Addition of "*with the exception of abnormal troponin values*" in Exclusion criteria 17.
6.10.1	Addition of troponin T level as a clinical evaluation of myocarditis.
7.1.3	Clarification on assessment in early terminated subjects including clarification on collection of AEs, MAAEs and SAEs due to trial pause.
7.1.4	Clarification on the dosing schedule in Cohorts 1 to 9 subjects due to trial pause.
7.2 and 8.4.1	Clarification on collection of AEs, MAAEs and SAEs due to trial pause.
7.4	Clarification on replacement of discontinued trial subjects due to trial pause.
8.6.2	Deletion of BNT165e assessment on sporozoite activity from additional research analysis.

10.9.7 Protocol update from version 7.0 to 8.0

The amendment (updating from version 7.0 to 8.0) adds information on the follow-up of treatment-discontinued subjects.

Other changes were clarifications (see the below tabular summary).

Detailed description of changes

Minor editorial changes, such as rewording or improvement of language for consistency, are not specifically listed.

Section	Reason for change
Tittle page	Sponsor signatory name has been updated.
1.1 and 3	Clarification on secondary objective, i.e., inclusion of "*geometric means and 95% CI*".
1.1 and 3	Primary objective: change in MAAEs and SAEs collection timepoint to make the outcome measure applicable to those subjects who prematurely discontinued IMP.
1.3	Clarification on visit descriptions for Dose 2 and Dose 3 in Tables 2 and 3.
1.3	Deletion of footnote "*n*" in Table 2 and "*m*" in Table 3 and clarification on Tables 2 and 3 notes to perform safety follow up after Dose 1 and to complete all assessments per protocol after Dose 2.
7.1	Clarification on follow-up of subjects who did not complete all doses.
7.2	Deletion of text on subject withdrawal and referred to Section 7.1.3 to avoid duplication.
7.1.3	Clarification to perform safety follow up after Dose 1 and to complete all assessments per protocol after Dose 2.
8.4.1	Clarification on collection of AE and SAE information.
9.5.2.1	Addition of SAEs and MAAEs analyses timepoint.
10.4.4	Deletion of "Additional Information and Follow-up Form" information.

11 REFERENCES

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12 TOXICITY MANAGEMENT/MITIGATION PLANS

12.1 Management of risks associated with anemia

This trial limits the total volume of blood drawn per subject over each sequential 8 week period to less than 550 mL, however, if a trial subject develops anemia, they should be evaluated and treated with iron supplements if appropriate.

If a trial subject develops Grade 3 anemia, the volume of blood drawn at any visit will be kept to a minimum, i.e., may be reduced to only safety assessments, at the discretion of the investigator.

13 APPENDICES

13.1 Appendix 1

ECG findings of potential clinical concern that **may** qualify as AEs:

- Marked sinus bradycardia (rate <40 bpm) lasting >2 minutes;
- New PR interval prolongation >280 ms;
- New prolongation of QTcF to >480 ms (absolute) or by ≥60 ms from baseline;
- New-onset atrial flutter or fibrillation, with controlled ventricular response rate (i.e., rate >120 bpm);
- New-onset Type I second-degree (Wenckebach) AV block of >30 seconds duration;
- Frequent premature ventricular contractions, triplets, or short intervals (<30 seconds) of consecutive ventricular complexes.

ECG findings that **may** qualify as SAEs:

- QTcF prolongation >500 ms;
- New ST-t changes suggestive of myocardial ischemia;
- New-onset left bundle branch block (QRS complex >120 ms);
- New-onset right bundle branch block (QRS complex >120 ms);
- Symptomatic bradycardia;
- Asystole:
    - In awake, symptom-free subjects with sinus rhythm, with documented periods of asystole ≥ 3 seconds or any escape rate <40 bpm, or with an escape rhythm that is below the AV node.
    - In awake, symptom-free subjects with atrial fibrillation and bradycardia with one or more pauses of at least 5 seconds or longer.
    - Atrial flutter or fibrillation, with rapid ventricular response rate >120 bpm.
- Sustained (i.e., short duration with relevant symptoms, or lasting >1 minute) supraventricular tachycardia (rate >120 bpm);

- Ventricular rhythms >30 seconds duration, including idioventricular rhythm (rate <40 bpm), accelerated idioventricular rhythm (rate 40 bpm to <100 bpm), and monomorphic/polymorphic ventricular tachycardia (rate >100 bpm);
- Type II second-degree (Mobitz) AV block;
- Complete (third-degree) heart block.

ECG findings that qualify as SAEs:

- Change in pattern suggestive of new myocardial infarction;
- Sustained ventricular tachyarrhythmias (>30 seconds duration);
- Second- or third-degree AV block requiring pacemaker placement;
- Atrial flutter or fibrillation with rapid ventricular response requiring cardioversion;
- Ventricular fibrillation/flutter.