CLINICAL TRIAL PROTOCOL

Trial number: BNT166-01

Document version:	6.0	**Date:**	19 DEC 2024
Sponsor name:	BioNTech SE 55131 Mainz, Germany		
Trial title:	A randomized, partially observer-blind, dose-escalation, Phase I/II trial evaluating the safety and immunogenicity of investigational RNA-based mpox vaccine candidates		
Brief lay title:	A clinical study investigating the safety and immune responses after immunization with investigational monkeypox vaccines		
Trial phase:	Phase I/II		
Investigational medicinal product (IMP):	Multivalent vaccine candidates BNT166a and BNT166c		
Regulatory identifiers:	IND 29806; ClinicalTrials.gov identifier NCT05988203		
Trial sites:	Multiple sites in United Kingdom (UK) and the United States of America (US)		
Trial medical monitor:	For the name and contact details, see the Trial Contact List filed in the Investigator's Site File (ISF)		

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 and the person authorized to sign (approve) the protocol and any protocol amendment(s) for the sponsor.

Some sponsor tasks in the conduct of this trial may be delegated, e.g., to contract research organization (CRO) personnel. Documentation of any delegation of responsibilities will be maintained.

Document history	Date	Version number
First sponsor approved version	20 MAR 2023	1.0
Second sponsor approved version	14 JUL 2023	2.0
Third sponsor approved version reflecting FDA feedback	08 SEP 2023	3.0
Fourth sponsor approved version	26 MAR 2024	4.0
Fifth sponsor approved version	13 MAY 2024	5.0
Sixth sponsor approved version	19 DEC 2024	6.0

See Section 10.8 for rationales/summaries of all previous protocol updates.

Statement of Compliance: This trial will be conducted according to this protocol, the ethical principles that have their origin in the Declaration of Helsinki, good clinical practice (GCP), and applicable regulatory requirements.

1 PROTOCOL SUMMARY

1.1 Synopsis

Trial title

A randomized, partially observer-blind, dose-escalation, Phase I/II trial evaluating the safety and immunogenicity of investigational RNA-based mpox vaccine candidates

Brief lay title

A clinical study investigating the safety and immune responses after immunization with investigational monkeypox vaccines

Trial phase Phase I/II

Regulatory identifiers IND 29806; ClinicalTrials.gov identifier NCT05988203

Trial rationale

Monkeypox (mpox) rapidly developed into a multinational outbreak beginning in May 2022 (WHO Interim Guidance for monkeypox). Low levels of transmission of this zoonotic infection have supported endemicity in West and Central Africa for years, but there was brisk community spread in non-endemic regions including Europe and North America (WHO Global Trends 2023; Gessain et al. 2022; Thornhill et al. 2022). In July 2022, the World Health Organization (WHO) declared the mpox outbreak to be a public health emergency of international concern (PHEIC; WHO Emergency Committee Meeting 2022; Nuzzo et al. 2022). Even more recently, different clades of the same MPXV, termed clades Ia and clade Ib, have driven an ongoing outbreak in the Democratic Republic of the Congo (DRC) and neighboring countries such as Burundi, Rwanda, Kenya, and Uganda. Due to the rising number of mpox cases and spread into other countries, the WHO on 14 AUG 2024 declared mpox a PHEIC, the second time this declaration has been made in the past 2 years. The Africa Centers for Disease Control and Prevention (Africa CDC), having been given the mandate by the African Union, has also declared mpox a Public Health Emergency of Continental Security – the health organization's first-ever declaration of a regional health emergency (Gostin et al. 2024). These declarations highlight the importance of developing safe, efficacious, and scalable medical countermeasures to prevent further worsening of the current outbreak.

The goal of the international outbreak response is to halt human-to-human transmission of the MPXV and prevent onward spread of disease, with a priority focus on communities at high risk of exposure (WHO Guidance 2022). Although there are two vaccines (MVA-BN and LC16) that already received emergency use authorization against mpox in the DRC, supplies of these vaccines are limited (Rigby 2024). There is also only limited preliminary clinical effectiveness data available for their use against mpox (CDC 2023). Thus, the development of mpox vaccines with improved immunogenicity and a favorable safety profile is needed, especially newer generation vaccines that can be rapidly manufactured at scale and easily adapted to address emerging new strains of the MPXV and other infectious agents.

Variola virus, a closely related Orthopoxvirus, is a Category A bioterrorism agent and can pose a formidable threat given the current limitations in supply of canonically produced vaccines, especially in the context of a largely unvaccinated world population after smallpox eradication in 1980 (Darling et al. 2002; Lum et al. 2022). This waning population-level immunity against

Orthopoxviruses is partly responsible for the current mpox outbreak which, with growing globalization and deforestation, may only be a harbinger for the next Orthopoxvirus emergence (Reynolds and Damon 2012; WHO Press Conference 27 JUL 2022). In preparation for this scenario, BioNTech plans to study if a ribonucleic acid (RNA)-based vaccine can be used to immunize against Orthopoxviruses. Given the high level of sequence identity between variola and mpox viruses, a vaccine against mpox may already cross-protect against other Orthopoxviruses including variola virus. RNA-based vaccines can also be rapidly modified in the event of novel Orthopoxvirus emergence.

To develop such an improved mpox vaccine, BioNTech is using the RNA technology platform used for the BNT162b2 severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) vaccine "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) are unchanged. Essentially, only the open reading frames are changed and now encode MPXV antigens.

The MPXV, which belongs to the Orthopoxvirus genus, has two forms in its viral lifecycle, mature virions (MVs) and extracellular virions (EVs). Each of these viral forms has a distinct set of surface antigens. Therefore, to effectively reduce virus replication and infectivity, the BNT166 multivalent vaccine will target MPXV proteins expressed in MVs and EVs. The final BNT166 multivalent mpox vaccine selected for Phase II/III development will consist of a combination of LNP-encapsulated RNAs encoding different MPXV antigens.

Overall design

BNT166 clinical development will begin with this Phase I/II first-in-human (FIH), randomized, partially observer-blind and sponsor-unblinded dose-escalation clinical trial designed to assess the safety, tolerability, reactogenicity, and immunogenicity of different multivalent vaccine candidates (BNT166a and BNT166c) in healthy adults.

This trial includes four substudies (see below and Table 1):

- Substudy A is an open-label, dose-escalation, Phase I substudy to assess the reactogenicity, safety, and immunogenicity of up to three dose levels of BNT166a and one dose level of BNT166c. This substudy will enroll ~64 healthy subjects with no prior history of known or suspected smallpox vaccination* (i.e., vaccinia-naïve subjects). The sponsor has decided not to activate Group A3, which was planned to be dosed with vaccine candidate BNT166c.
- Substudy B is a randomized, observer-blinded and sponsor-unblinded, Phase I substudy to assess the reactogenicity, safety, and immunogenicity of one dose level of BNT166a and BNT166c. This substudy will enroll ~32 healthy subjects with prior history of smallpox vaccination* (vaccinia-experienced subjects). The sponsor may decide to not activate one of the groups, in that case this substudy will be a one group open-label substudy. Accordingly, the sponsor has decided not to activate Group B2, which was planned to be dosed with vaccine candidate BNT166c.
- Substudy D is an open-label, Phase IIa substudy to assess the reactogenicity, safety, and immunogenicity of one dose level of BNT166a. This substudy will enroll ~32 healthy subjects with no prior history of known or suspected smallpox vaccination* (i.e., vaccinia-naïve subjects).

* The smallpox vaccination status will be determined based on the medical record of vaccination before 1980 or the presence of the smallpox vaccination characteristic scar.

Safety and reactogenicity will be evaluated further at the timepoints indicated in the respective substudy schedule of activities (SoA) (Sections 13.3.4 for Substudy A, 14.3.4 for Substudy B, and 15.3.4 for Substudy D).

The duration of trial participation will be ~14 months per subject in all of the substudies (including a ≤28 d screening period prior to Visit 1, and 12–14 visits on site spread over ~13 months).

Depending on the safety and immunogenicity data generated in this trial, some substudies/groups may not be initiated or may be terminated earlier. Protocol amendments may be used to allow other substudies or groups to be initiated and/or for other multivalent vaccine candidates to be tested.

Objectives, outcome measures, and endpoints

For the objectives, outcome measures, and endpoints of each substudy see Section 13.4 for Substudy A, Section 14.4 for Substudy B, and Section 15.4 for Substudy D.

The primary objectives for all substudies are to describe the reactogenicity and safety after one and two doses of each multivalent vaccine candidate.

Trial treatments

For an overview of the treatment groups and trial treatments, see Table 1. For details about the IMPs in each substudy, see Section 13.6 for Substudy A, Section 14.6 for Substudy B, and Section 15.6 for Substudy D.

Table 1: Overview of treatment groups, populations, and trial treatments

Substudy	Group	Population [a]	IMP	Dose	Dosing route	No. of subjects	Blinding
A	A1	Vaccinia-naïve	BNT166a	10 µg	IM	16	Open-label
	A2	Vaccinia-naïve	BNT166a	30 µg	IM	16	
	A3	Vaccinia-naïve	BNT166c	30 µg	IM	16	
	A4	Vaccinia-naïve	BNT166a	60 µg	IM	16	
B	B1	Vaccinia-experienced	BNT166a	30 µg	IM	16	Observer-blind [c]
	B2	Vaccinia-experienced	BNT166c	30 µg	IM	16	
D	D1	Vaccinia-naïve	BNT166a	60 µg [b]	IM	32	Open-label

a) Vaccinia-naïve = no prior history of known or suspected smallpox vaccination. Vaccinia-experienced = medical record of vaccination before 1980 or the presence of the smallpox vaccination characteristic scar.

b) The dose was selected based on Substudy A and Substudy B data.

c) The sponsor may decide to not activate one of the groups, in that case this substudy will be a one group open-label substudy.

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

Dosing scheme for Substudies A, B, and D

This trial will use a staggered dosing process including dose-escalation for Substudy A, and a staggered dosing process for Substudy B. For a graphical depiction of the dosing process for all substudies, see Figure 1. For detailed descriptions of the process in each substudy, see Section 13.3.1 for Substudy A, Section 14.3.1 for Substudy B, and Section 15.3.1 for Substudy D.

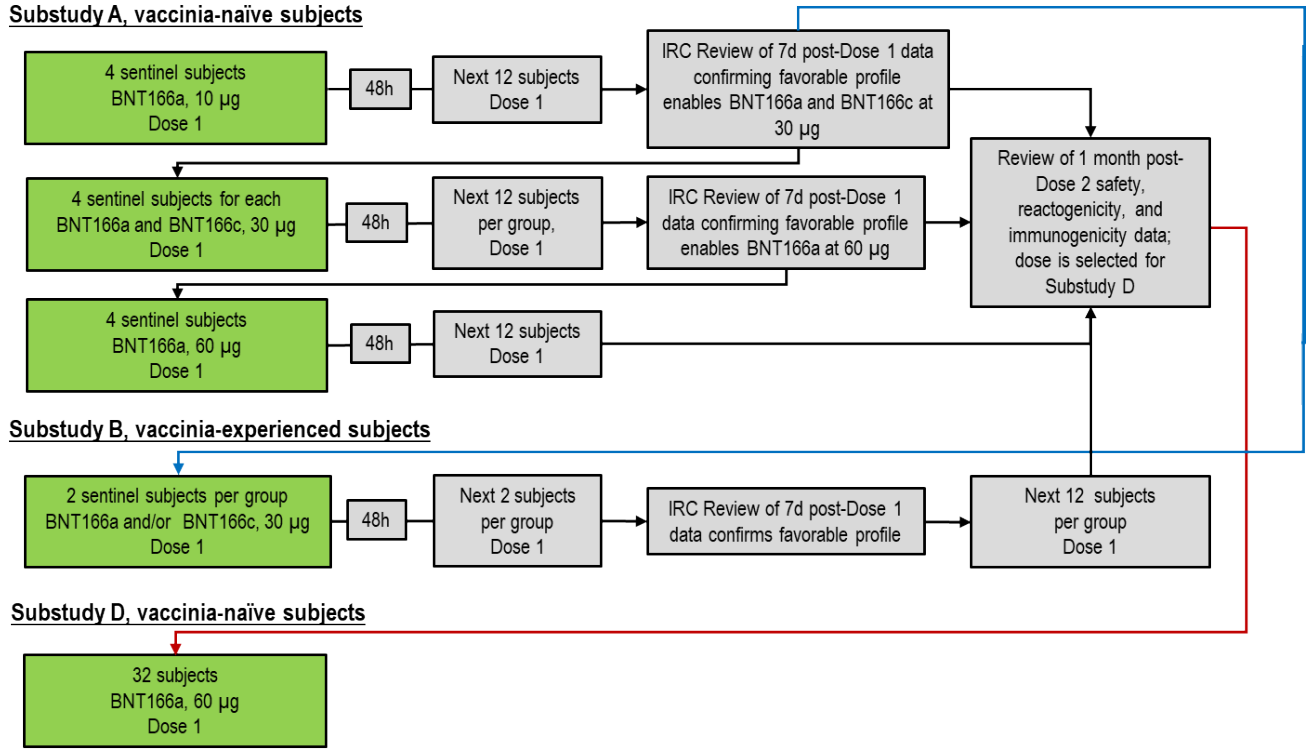

Figure 1: The dosing process for Substudies A, B, and D

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

Note: The blue line indicates that the start of Substudy B is dependent on IRC confirmation of a favorable safety profile in Substudy A. The red line indicates that the start of Substudy D and the dose administered in this substudy is dependent on a review of data from Substudies A and B.

Statistics

No formal sample size calculations have been performed. The sample size for each group is mainly driven by typical FIH Phase I designs for the 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 an IMP. The probability of observing at least one adverse event (AE) for a given true event rate of 10% is 81.5% in 16 subjects per group in Substudies A and B. For Substudy D, the probability of observing at least one AE for a given true event rate of 10% is 96.6% in 32 subjects. The sample size of 32 subjects in Substudy D has been chosen to provide supporting information regarding the immunogenicity of the IMP.

No formal statistical testing will be done. In general, descriptive summary tables and figures will be presented by treatment group for each substudy per multivalent vaccine candidate. Where

appropriate, data will also be summarized overall across substudies per multivalent vaccine candidate.

Continuous variables will be summarized by treatment group using the following descriptive statistics: number of subjects (n), mean, standard deviation, median, minimum and maximum, unless otherwise stated.

Categorical variables will be summarized by group presenting absolute and relative frequencies (n and %) of subjects in each category. Baseline is defined as last available value prior to first IMP administration. Where appropriate, changes from baseline (pre-Dose 1) will be assessed.

All safety analyses, including those for the primary objectives of the trial, will be based on the Safety Set (i.e., all subjects who received at least one dose of IMP). All immunogenicity analyses will be based on the Immunogenicity Analysis Sets specified in Section 9.4.

Reactogenicity data grading will be based on the United States Food and Drug Administration (US FDA) guidance (US FDA 2007). All AEs will be coded to a system organ class and preferred term using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA) coding system.

1.2 Schema

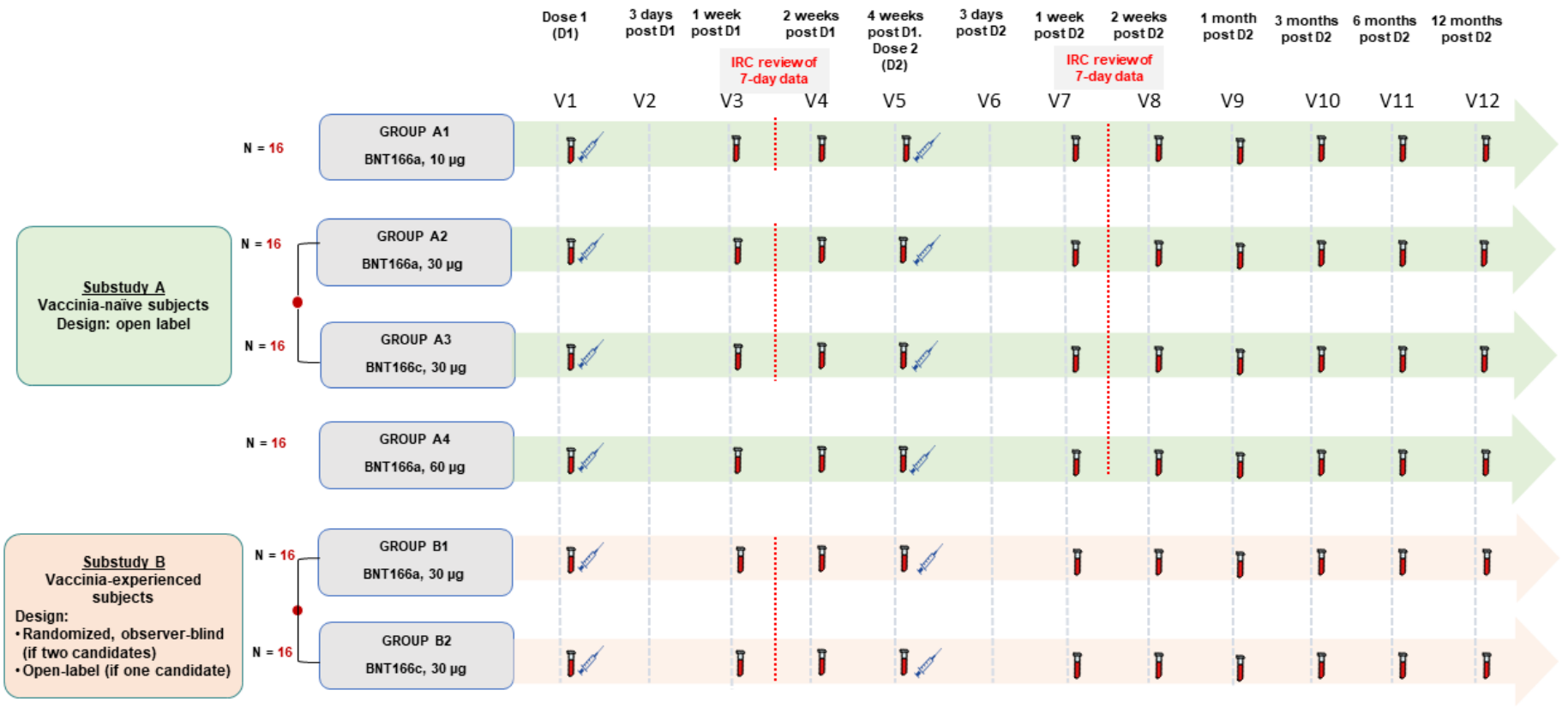

Figure 2: Graphical representation of the trial – Substudies A and B

Notes: Syringes indicate vaccination. Blood vials indicate blood draws.

Abbreviations: D = dose; IRC = Internal Review Committee; N = number of subjects; V = visit.

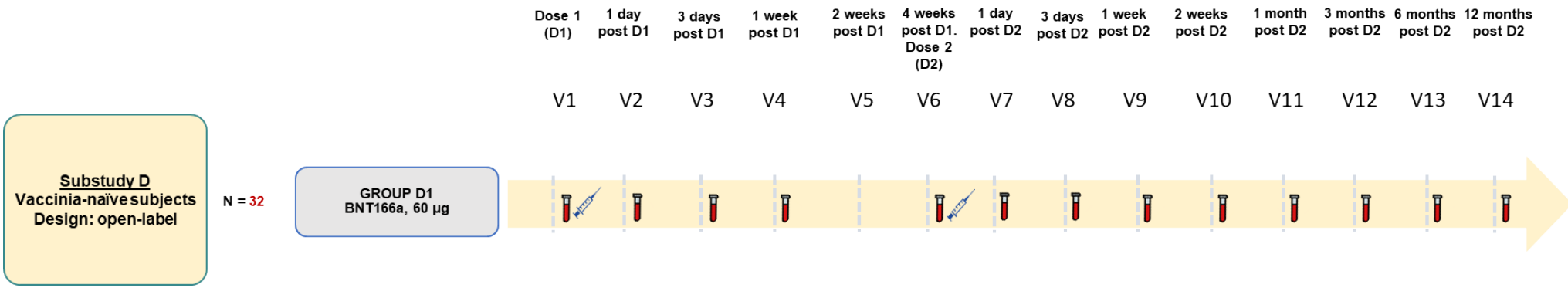

Figure 3: Graphical representation of the trial – Substudy D

Notes: Syringes indicate vaccination. Blood vials indicate blood draws.
Abbreviations: D = dose; N = number of subjects; V = visit.

1.3 Schedule of activities

For SoAs for each substudy, see Section 13.3.4 for Substudy A, Section 14.3.4 for Substudy B, and Section 15.3.4 for Substudy D.

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ABBREVIATIONS/TERMS

For standard definitions, see Section 10.7.

Abbreviation/Term	Explanation
~	Approximately
AE	Adverse event
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
ALT	Alanine transaminase
anti-HCV	Anti-Hepatitis C virus antibodies
AST	Aspartate transaminase
CRF	Case report form
CI	Confidence interval
CRO	Contract research organization
CSR	Clinical study report
d	Day
DILI	Drug induced liver injury
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EDC	Electronic data capture (system)
EU	European Union
EV	Extracellular virion
FIH	First-in-human
FSH	Follicle stimulating hormone
GCP	Good clinical practice
h	Hour
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HRT	Hormonal replacement therapy
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Council for Harmonisation (of Technical Requirements for Pharmaceuticals for Human Use)
ICMJE	International Committee of Medical Journal Editors

Abbreviation/Term	Explanation
IEC	Independent Ethics Committee
IM	Intramuscular or intramuscularly
IMP	Investigational medicinal product
IRB	Institutional Review Board
IRC	Internal Review Committee
ISF	Investigator's Site File
LNP	Lipid nanoparticle
MAAE	Medically attended adverse event
MedDRA	Medical Dictionary for Regulatory Activities
mpox	Monkeypox
MPXV	Monkeypox virus
MV	Mature virion
MVA-BN	Modified Vaccinia Ankara - Bavarian Nordic
NSAID	Non-steroidal anti-inflammatory drug
PCR	Polymerase chain reaction
PRNT	Plaque reduction neutralization test
QT	The time between the start of the QRS complex and the end of the T wave in an ECG
QTcF	Corrected QT interval by Fridericia
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
SoA	Schedule of activities
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TMF	Trial Master File
TOPS	(UK) Over-Volunteering Prevention System
UK	United Kingdom
ULN	Upper limit of normal
US FDA	United States Food and Drug Administration
VOCBP	"Volunteers" of childbearing potential is used instead of "women" of childbearing potential to encompass all volunteers born female
WHO	World Health Organization

2 INTRODUCTION

2.1 Trial rationale

Mpox rapidly developed into a multinational outbreak beginning in May 2022 (WHO Interim Guidance for monkeypox). Low levels of transmission of this zoonotic infection have supported endemicity in West and Central Africa for years, but there was brisk community spread in non-endemic regions including Europe and North America (WHO Global Trends 2023; Gessain et al. 2022; Thornhill et al. 2022). In July 2022, the WHO declared the mpox outbreak to be a PHEIC (WHO Emergency Committee Meeting 2022; Nuzzo et al. 2022). Even more recently, different clades of the same MPXV, termed clades Ia and clade Ib, have driven an ongoing outbreak in the DRC and neighboring countries such as Burundi, Rwanda, Kenya, and Uganda. Due to the rising number of mpox cases and spread into other countries, the WHO on 14 AUG 2024 declared mpox a PHEIC, the second time this declaration has been made in the past 2 years. The Africa CDC, having been given the mandate by the African Union, has also declared mpox a Public Health Emergency of Continental Security – the health organization's first-ever declaration of a regional health emergency (Gostin et al. 2024). These declarations highlight the importance of developing safe, efficacious, and scalable medical countermeasures to prevent further worsening of the current outbreak.

The goal of the international outbreak response is to halt human-to-human transmission of the MPXV and prevent onward spread of disease, with a priority focus on communities at high risk of exposure (WHO Guidance 2022). Although there are two vaccines (MVA-BN and LC16) that already received emergency use authorization against mpox in the DRC, supplies of these vaccines are limited (Rigby 2024). There is also only limited preliminary clinical effectiveness data available for their use against mpox (CDC 2023). Thus, the development of mpox vaccines with improved immunogenicity and a favorable safety profile is needed, especially newer generation vaccines that can be rapidly manufactured at scale and easily adapted to address emerging new strains of the MPXV and other infectious agents.

Variola virus, a closely related Orthopoxvirus, is a Category A bioterrorism agent and can pose a formidable threat given the current limitations in supply of canonically produced vaccines, especially in the context of a largely unvaccinated world population after smallpox eradication in 1980 (Darling et al. 2002; Lum et al. 2022). This waning population-level immunity against Orthopoxviruses is partly responsible for the current mpox outbreak which, with growing globalization and deforestation, may only be a harbinger for the next Orthopoxvirus emergence (Reynolds and Damon 2012; WHO Press Conference 27 JUL 2022). In preparation for this scenario, BioNTech plans to study if an RNA-based vaccine can be used to immunize against Orthopoxviruses. Given the high level of sequence identity between variola and mpox viruses, a vaccine against mpox may already cross-protect against other Orthopoxviruses including variola virus. RNA-based vaccines can also be rapidly modified in the event of novel Orthopoxvirus emergence.

To develop such an improved mpox vaccine, BioNTech is using the RNA technology platform used for the BNT162b2 SARS-CoV-2 vaccine "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) are unchanged. Essentially, only the open reading frames are changed and now encode MPXV antigens.

The MPXV, which belongs to the Orthopoxvirus genus, has two forms in its viral lifecycle, MVs and EVs. Each of these viral forms has a distinct set of surface antigens. Therefore, to effectively reduce virus replication and infectivity, the BNT166 multivalent vaccine will target MPXV proteins expressed in MVs and EVs. The final BNT166 multivalent mpox vaccine selected for Phase II/III development will consist of a combination of LNP-encapsulated RNAs encoding different MPXV antigens.

BNT166 clinical development will begin with this Phase I/II FIH, randomized, partially observer-blind and sponsor-unblinded dose-escalation clinical trial designed to assess the safety, tolerability, reactogenicity, and immunogenicity of different multivalent vaccine candidates (BNT166a and BNT166c) in healthy adults.

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

2.2 Background

2.2.1 The medical need

Since early May 2022, cases of mpox were reported from countries where the disease is not endemic, and continued to be reported in several endemic countries. Even more recently, different clades of the same MPXV have driven an ongoing outbreak in the DRC and neighboring countries such as Burundi, Rwanda, Kenya, and Uganda, which was declared a PHEIC by the WHO.

Mpox is caused by an enveloped, double-stranded deoxyribonucleic acid (DNA) virus of the Orthopoxvirus genus of the Poxviridae family. Infection with mpox classically progresses through three stages: incubation, prodromal and eruptive (during which the classic rash develops and progresses). Mpox is usually a self-limited disease with clinical manifestations lasting around 4 weeks. Complications include bacterial superinfections of the rash (occurring 3 to 4% of the time during the current outbreak), pneumonia and encephalitis (Patel et al. 2022; Tarín-Vicente et al. 2022). If the rash involves an eye, corneal ulceration or keratitis can occur, both of which carry a considerable risk of imminent vision loss (Gessain et al. 2022; Cash-Goldwasser et al. 2022; Badenoch et al. 2022). During the 2022/2023 outbreak, ocular complications have occurred ~1 to 3% of the time, but during prior outbreaks in endemic regions ocular complications have occurred more frequently (Kaufman et al. 2023). During an earlier outbreak in the Democratic Republic of Congo for example, conjunctivitis alone occurred ~23% of the time and keratitis occurred in 3 to 4% of mpox cases (Hughes et al. 2014).

Newborn babies, children, pregnant women and people with underlying immune deficiencies are at a higher risk of more severe disease, complications, and death from mpox. In the past, the case fatality ratio of mpox ranged from 0 to 11% in the general population and has been higher in young children (WHO Fact Sheet April 2023; Thornhill et al. 2022; Adler et al. 2022). The death rates in different settings may differ due to factors such as access to health care. Globally in 2022–2024, there have been 203 deaths due to

mpox reported by 31 MAY 2024 (WHO Global Trends 2024). Notably, in an ongoing outbreak in the DRC, a total of 22,500 suspected mpox cases including over 1,000 deaths (case fatality ratio 4 to 5%) have been reported from 01 JAN 2023 through end of May 2024 (WHO 2024). Among suspected cases, ~10% have been tested by polymerase chain reaction (PCR), and ~65% were positive for MPXV.

Currently, only one vaccine (non-replicating, live, attenuated, third generation vaccine based on the MVA-BN) is authorized in the European Union (EU) and the US for mpox prevention (trade name JYNNEOS in US, IMVANEX in EU, and IMVAMUNE in Canada). In 2013, IMVANEX was authorized in the EU for active immunization against smallpox under exceptional circumstances. The indication “active immunization against mpox in adults” was added in July 2022 following the current outbreak. In contrast to this, JYNNEOS was initially authorized in the US in 2018 for both mpox as well as smallpox prevention (on request of the US Center for Biologics Evaluation and Research [CBER]).

MVA-BN is indicated in individuals 12 years of age and older determined to be at high risk for smallpox or MPXV infection. MVA-BN is licensed as a course of two doses given subcutaneously at least 28 d apart. Additionally, the EU and the FDA now allow for a lower dose (20%) of the MVA-BN dose to be administered intradermally to increase the number of individuals who could be dosed with the limited supply. Two doses of the vaccine are still needed. Although approved for use against mpox, MVA-BN supplies are limited and recent publications reported that a two-dose MVA-BN immunization series in non-primed individuals may yield relatively low levels of MPXV-neutralizing antibodies (Zaeck et al. 2023; Bertran et al. 2023).

A second-generation vaccine, ACAM2000, is a live replication-competent vaccine. ACAM2000 was first approved by the FDA in 2007 for active immunization for the prevention of smallpox disease in individuals determined to be at high risk for smallpox infection. Recently, in August 2024, the FDA-approved ACAM2000 for prevention of mpox disease in individuals determined to be at high risk for mpox infection (Drugs.com 2024). ACAM2000 does not provide an advantageous safety profile when compared with third-generation vaccines and is not authorized in the EU.

There is limited data currently available on the clinical efficacy or effectiveness of MVA-BN or ACAM2000 vaccines for the prevention of mpox (IMVANEX EPAR EMA/657731/2022; Bertran et al. 2023; Zaeck et al. 2023; Mason et al. 2024). Preliminary JYNNEOS vaccine effectiveness estimates against medically attended mpox disease in the US have been published (CDC 2023). JYNNEOS vaccine is effective at reducing the risk of mpox disease, with two doses providing the best protection, regardless of the route of vaccine administration.

Currently in the DRC, there are two vaccines (MVA-BN and LC16) that have received emergency use authorization against mpox, however supplies of these vaccines are limited (Rigby 2024). There is also only limited preliminary clinical effectiveness data available for their use against mpox (CDC 2023). Thus, the development of mpox vaccines with improved immunogenicity and a favorable safety profile is needed, especially newer generation vaccines that can be rapidly manufactured at scale and easily adapted to address emerging new strains of the MPXV and other infectious agents.

2.2.2 Clinical development of a BioNTech multi-antigen vaccine

The objective of the BNT166 multivalent vaccine program is to develop a vaccine with proven effectivity, improved safety, and improved durability, which can be rapidly manufactured at scale.

As demonstrated by the SARS-CoV-2 vaccine BNT162b2 “COMIRNATY”, the development of vaccines with RNA-LNPs encoding viral antigens that are translated to protein by the vaccinated organism to induce a protective immune response provides significant benefits, including:

- **Safety**: The available smallpox and mpox vaccines are live whole virus vaccines. Based on the current understanding of the safety experience with BNT162b2, mRNA-based vaccines are expected to have a better safety profile than whole virus vaccines both in the general population and in special populations like the immunocompromised individuals.
- **Production**: MVA-BN, a heavily attenuated viral vaccine based on Modified Vaccinia Ankara, does not replicate and must therefore be given at a high dose to achieve satisfactory immunogenicity. The required high dose and the complex/time consuming manufacturing process required for MVA-BN is not suitable for rapid large scale production. In contrast, RNA-based vaccine production does not require individualized cell culture systems and unique purification systems for each vaccine antigen. BioNTech’s experience with COMIRNATY has shown that RNA-based vaccines can be manufactured rapidly at a scale for population-level vaccination if required.
- **Multi-antigen vaccines**: Orthopoxviruses are known to take two forms in the viral lifecycle (MVs and EVs). Each of these forms expresses a different set of surface antigens, and therefore to reduce viral replication and infectivity, it is presumed that antibodies to proteins from both forms are needed. 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 a multivalent modRNA-based mpox vaccine that will contain at least one target from each of the two lifecycle forms.

Clinical development of the BioNTech multivalent vaccine candidates will begin with this Phase I/II FIH clinical trial designed to assess the safety, tolerability, reactogenicity and immunogenicity of different multivalent vaccine candidates (BNT166a and BNT166c) with different combinations of RNAs encoding different MPXV antigens.

This trial will be initiated with the multivalent vaccine candidates BNT166a and BNT166c (see below for the mpox antigens targeted by each included monovalent drug product):

- BNT166a: antigens B6, A35, M1, and H3 (component ratio of 1:1:1:1).
- BNT166c: antigens B6, A35, and M1 (component ratio of 1:1:1).

For more details about the BNT166 multivalent vaccine candidates, see the BNT166 IB. The final BNT166 multivalent mpox 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

For further information on the expected benefits and risks connected with intramuscular (IM) administration of the BNT166 multivalent vaccine candidates, see Sections 13.2.3 (Substudy A), 14.2.3 (Substudy B), 15.2.3 (Substudy D), and the BNT166 IB (which includes the reference safety information).

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 mpox and smallpox vaccines, and thereby may also help the development of a safe and effective prophylactic vaccine for active immunization against mpox and smallpox.

The potential benefits of receiving one of the BNT166 multivalent vaccine candidates in this trial have not been demonstrated, therefore subjects may have no benefit from receiving this trial treatment, but by undergoing laboratory tests and physical examinations in this trial, previously undiagnosed health problems may be uncovered.

2.3.2 Risk assessment

The general risks of vaccines are included in Section 7.2.1 of the BNT166 IB.

Potential risks related to administration of the multivalent vaccine candidates:

Human experience with the BNT166 multivalent vaccine candidates is not available prior to this trial. These vaccine candidates include RNA, which is metabolized just like other RNA in humans and does not interfere with the DNA of a cell. The RNA, which is delivered to the cells of the vaccinated host, allows expression of BNT166 antigens and elicits an immune response that ideally disrupts the mpox and smallpox viruses' lifecycle at different stages, and thus prevents clinical manifestation of mpox and smallpox.

Data from non-clinical studies and clinical trials with the same RNA vaccine platform (including for BNT162b2 "COMIRNATY") support a favorable benefit/risk profile. Based on risks observed with SARS-CoV-2 vaccine BNT162b2 (with the same LNP carrier system, the same N1-methylpseudouridine modified [i.e., nucleoside-modified] RNA, and the same untranslated elements of the antigen-encoding RNA), the safety profile for BNT162b2 summarized in the BNT166 IB may suggest similar risks after BNT166 multivalent vaccine candidate administration.

For a detailed risk assessment of the BNT166-encoded antigens, see Section 5.3.1 of the BNT166 IB.

Potential risks related to trial procedures:

- Trial subjects will be required to attend healthcare facilities during the trial and thus there is a potential for increased exposure to SARS-CoV-2 and other respiratory infections like influenza.
- Venipuncture will be performed during the trial. There is the risk of bleeding, bruising, hematoma formation, and infection at the venipuncture site.

2.3.3 Risk mitigation

General risks of vaccines and potential risks related to trial procedures are managed by i) the use of sites experienced with vaccinations, ii) the use of sites experienced with trials of this type, and iii) the use of suitably trained and experienced site personnel.

Potential risks related to administration of trial treatment and/or trial procedures are managed by:

- Safety monitoring after each dose, including performing subject observation for at least 1 h after each IMP administration, regular health checks (symptom-directed physical examination, vital signs), real time monitoring of injection site reactions and systemic events via subject e-diaries, daily investigator review of subject's e-diary data, and wellbeing phone calls.
- Ensuring that equipment and medication to treat anaphylaxis is readily available.
- Assessment of clinical chemistry and hematology parameters at screening, on the dosing days, and at ~1 week and ~1 month after each IMP administration.
- Electrocardiogram (ECG) recordings before IMP administration, on dosing days and at Day 3, ~1 week and ~2 weeks each IMP administration.
- Close monitoring of AEs collected during ~1 month after each IMP administration; expedited reporting of serious adverse events (SAEs) by the trial sites for 1 year after the last IMP administration.
- Use of sentinel subjects/staggered dosing. Dosing in Substudies A and B will start with an initial sentinel group, and thereafter continue using a staggered dosing approach.
- Use of pausing rules. Substudy A will investigate escalating doses, and only allow progression to the next dose level if no pausing rules are met.
- Use of a trial Internal Review Committee (IRC) to review the safety data at prespecified timepoints and at any timepoint if there are any safety concerns, see Section 7.1. If required, the trial IRC may decide to unblind the trial treatment for affected subject(s), and/or to stop IMP administration to other subjects in a particular group.
- Requiring the trial medical monitor and safety physician to review the accumulating safety data on an ongoing basis, follow-up on pausing rules, and to interact/advise trial sites as needed.

For guidance on the management of risks associated with:

- Potential cases of drug induced liver injury (DILI; Section 10.6.1).
- Assessment of change in kidney function and detection of kidney injury (Section 10.6.2).
- Potential risks related to trial treatment administration, see the toxicity management/mitigation plans for local and systemic reactogenicity (Section 12.1).
- Anemia related to the blood draws in this trial, see Section 12.2.
- Potential increased exposure to SARS-CoV-2, and thus linked to the pandemic COVID-19, see Section 12.3.

2.3.4 Overall benefit/risk conclusion

The potential risk to subjects participating in this trial is considered as acceptable. The trial was designed to minimize risk to subjects, thus the potential risks identified in association with the trial procedures and treatments are considered justified by the anticipated benefits if a vaccine for active immunization against mpox and smallpox is successfully developed.

3 OBJECTIVES AND ENDPOINTS

For the substudy objectives and endpoints, see Sections 13.4 (Substudy A), 14.4 (Substudy B), and 15.4 (Substudy D).

4 TRIAL DESIGN

4.1 Overall design

This is a randomized, partially observer-blind, dose-escalation, Phase I/II trial evaluating the safety, tolerability, reactogenicity and immunogenicity of investigational RNA-based multivalent vaccine candidates for active immunization against mpox.

This trial includes four substudies. Each substudy design is described separately in the respective substudy section, see Sections 13.3 (Substudy A), 14.3 (Substudy B), and 15.3 (Substudy D).

Some substudies may be conducted in parallel, as required by the clinical plan, within the framework of this master protocol.

4.1.1 Additional description of the trial design

Not applicable.

4.1.2 Duration of the trial periods

The substudies in this trial comprise an ~4-week screening period and then an ~56 week assessment period with two visits with IMP administration and follow-up for up to 12 months after the last IMP dose.

4.1.3 Planned number of trial subjects

For the planned number of trial subjects, see Table 1.

4.2 Rationale for the trial design

For the rationales supporting the design of each substudy, see Sections 13.3 (Substudy A), 14.3 (Substudy B), and 15.3 (Substudy D).

The rationale for the randomized observer-blind trial design in Substudy B is to minimize selection bias and maximize the integrity of the safety/reactogenicity assessment.

Because of the exploratory nature of the BNT166-01 trial, no formal sample size calculation was performed for any of the groups. The sample size for each group is mainly driven by typical FIH Phase I designs for the 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 an IMP. For further details, see Section 9.3.

Since the BNT166 multivalent vaccine candidates will be evaluated for the first time in humans, this trial will be initiated with the dose-escalation Substudy A. Dosing in Substudies A and B will start with an initial sentinel group, pausing rules will be followed in Phase I substudies (Substudies A and B), 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 point of time. A staggered subject dosing will be utilized in Substudies A and B. For further details for each substudy, see Sections 13.3.1 (Substudy A), 14.3.1 (Substudy B), and 15.3.1 (Substudy D).

As a general rule, maximally eight subjects may be dosed per site per day in any substudy.

Since the BNT166 multivalent vaccine candidates will be evaluated for the first time in humans, this trial also includes the risk mitigation measures described in Section 2.3.2.

This trial uses safety evaluations and vaccine-induced immune responses to assess if there is a dose-response. For further details, see Section 8.7.

4.2.1 Subject input to the design

Subject input to the trial design was not obtained.

4.2.2 Rationale for the level of contraception

Currently, a risk of human teratogenicity/fetotoxicity cannot be excluded by available data. Therefore, all trial subjects who are biologically capable of having or fathering children must agree to use a highly effective method of contraception consistently (see Section 10.5.2) and correctly as specified in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]). In addition, pregnancy testing will be performed for Volunteers of childbearing potential (VOCBP) at screening and before each IMP administration.

For definitions of VOCBP, as well as guidance on how to collect pregnancy information, see Section 10.5.

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 scheduled procedure (final date on which data were or are expected to be collected) as shown in the SoA is completed for the last subject in the trial globally.

For definitions of the trial and site start/closure, see Section 10.7.1.

For guidance on trial discontinuation, see Section 10.1.12.

5 TRIAL POPULATION

The trial population 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 individual is suitable for enrollment.

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

Trial sites must ensure that compliance with the following inclusion/exclusion criteria are supported by the documentation, e.g., interview feedback, outcomes of trial procedures, medical records, etc., that confirm volunteers are eligible for inclusion in this trial.

For sites in the UK, all trial subjects will be registered in the UK Over-Volunteering Prevention System (TOPS) database.

5.1 Selection of trial population

For the populations in the different substudies, see Sections 13.5.1 (Substudy A), 14.5.1 (Substudy B), and 15.5.1 (Substudy D).

5.2 Rationale for trial population

For the rationale for the trial populations in the different substudies, see Sections 13.5.2 (Substudy A), 14.5.2 (Substudy B), and 15.5.2 (Substudy D).

5.3 Inclusion criteria

For the substudy inclusion criteria, see Sections 13.5.3 (Substudy A), 14.5.3 (Substudy B), and 15.5.3 (Substudy D). Some inclusion criteria are common to all substudies.

5.4 Exclusion criteria

For the substudy exclusion criteria, see Sections 13.5.4 (Substudy A), 14.5.4 (Substudy B), and 15.5.4 (Substudy D). Some exclusion criteria are common to all substudies.

5.5 Lifestyle considerations

Trial subjects will be required to remain at the site for at least 1 h after each IMP administration.

On visit days with blood collection and IMP administration, trial subjects will be asked to drink fluids (e.g., water) before the visit and during the visit per trial site standard.

Trial subjects will be asked to avoid strenuous or endurance exercise within 7 d before and after each IMP administration. Strenuous or endurance exercise is defined as any exercise above the standard level for a given subject.

Trial subjects will be required to follow the guidance from the trial site personnel on recommended social behaviors to avoid SARS-CoV-2 infection (e.g., mask wearing, social distancing) and to practice the prescribed forms of contraception (see Sections 8.4.5 and 10.5.2).

5.6 Screen failures

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

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 data, date the informed consent form (ICF) was signed, the reasons for screen failures, if applicable.

Individuals who do not meet the criteria for participation in this trial (screen failures) may be rescreened. Individuals who fail their first screening for trial eligibility may qualify for rescreening opportunities (for a total of three screenings per individual) at the investigator's discretion. Individuals must re-sign the ICF prior to each rescreening. Results of non-trial assessments performed prior to obtaining informed consent and within 28 d prior to the Visit 1 may be used; such assessments do not need to be repeated for screening or rescreening.

6 TRIAL TREATMENTS

Trial treatment is defined as any investigational treatments or marketed products intended to be administered to a trial subject according to this 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 administered

For an overall summary of the planned trial treatments in the substudies, see Table 1.

For details of the trial treatments in the substudies, see Sections 13.6 (Substudy A), 14.6 (Substudy B), and 15.6 (Substudy D).

Standard vaccination practices must be observed and the multivalent vaccine candidates must not be injected into blood vessels.

As with all injectable vaccines, trained medical personnel and appropriate medications and equipment should be readily available for management of anaphylactic/hypersensitivity reactions following vaccine administration.

6.2 Rationale for the trial treatment

The proposed dose levels for the BNT166 multivalent vaccine candidates in this trial are based on the safety and immunogenicity in 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 “PfizerBioNTech COVID-19 vaccine”. BNT162b2 and the BNT166 multivalent vaccine candidates 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 lowest dose for the BNT166 multivalent vaccine candidates in this trial is lower than the dose approved for BNT162b2 (10 µg versus 30 µg) and is also spread over more time, i.e., the primary two doses in the BNT162b2 trials were given 3 weeks apart, in this trial the two doses will be administered ~4 weeks (~31 d) apart.

The highest dose level for the BNT166 multivalent vaccine candidates in this trial (60 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.

The data for the 30 µg dose level for BNT162b2 in the Trial BNT162-01 were consistent with those of Trial C4591001, demonstrating appropriate immunogenicity. Therefore, the same dose level has been selected for the exploration of BNT166 multivalent vaccine candidates in vaccinia-experienced subjects in this trial. The immunogenicity of 30 µg in vaccinia-experienced subjects will be compared to that of vaccinia-naïve subjects to understand the impact of pre-existing immunity on vaccine response.

Since its first marketing authorization in December 2020, BNT162b2 (COMIRNATY) has been administered to hundreds of millions of individuals worldwide. Notably, these vaccines have been administered safely in pediatric, pregnant and immunocompromised populations, i.e., populations that are relevant to BNT166 development since these groups are at increased risk of severe mpox disease. 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.

6.3 Dosing and administration

6.3.1 Dose modifications

Dose modifications are not planned in this trial, but trial treatment may be temporarily paused or permanently discontinued. See Section 7.1 for guidance on such cases.

6.3.2 Temporary delaying of trial treatment administration for single trial subjects

In the following cases IMP administration should be delayed:

- Febrile illness (body temperature ≥38.0°C/≥100.4°F) or other acute illness, current and/or within 48 h before IMP administration.
- Receipt of influenza vaccine. IMP administration should be delayed to allow 14 d after influenza vaccine administration.

If any of the above events occur before the first IMP dose, the IMP allocation can be delayed within the allowed screening period at the discretion of investigator and after consultation with the sponsor medical monitor. If IMP allocation cannot occur within allowed screening period, the subject should be rescreened.

If any of the above events occur before the second IMP dose, the vaccination can be delayed, as long as this delay is within the allowed window (see the respective SoA). If the second IMP dose cannot be rescheduled within the allowed window, the trial medical monitor should be contacted for further guidance.

6.4 Actions in case of overdose or error 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:

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

Decisions regarding pausing or permanent discontinuation a subjects’ trial treatment will be made by the investigator in consultation with the trial medical monitor based on the clinical evaluation of the trial subject.

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.

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 the unblinded and authorized site personnel.

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.

The delegated unblinded site personnel, e.g., pharmacist or designee, is responsible for trial treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

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

6.6 Subject assignment, randomization, and blinding

Substudy A is an open-label, dose-escalation substudy. Substudy B is an observer-blind, randomized substudy. Substudy D is an open-label substudy.

6.6.1 Allocation of IMP

For allocation to IMP in the substudies, see Sections 13.3 (Substudy A), 14.3 (Substudy B), and 15.3 (Substudy D).

6.6.2 Blinding and unblinding

Substudy B is observer-blind. The sponsor may decide to not activate one of the groups in Substudy B. In that case Substudy B will be a one group open-label substudy. Further details relevant to blinding/unblinding procedures are provided in the Pharmacy Manual.

6.6.2.1 Blinding of trial subjects

Trial subjects in Substudies A and D will know their assigned trial treatment and dose. Trial subjects in Substudy B will be blinded to their assigned trial treatment. The sponsor may decide to not activate one of the groups in Substudy B, in that case trial subjects in Substudy B may become aware of their assigned trial treatment.

6.6.2.2 Blinding of trial site personnel

Substudies A and D are open-label substudies, site personnel will be aware of assigned treatment and dose.

Substudy B is observer-blind, such that only site personnel receiving, storing, dispensing, preparing, and administering the trial treatment will be unblinded. All other site personnel, including the investigator(s), investigator staff, will be blinded to subjects' assigned trial

treatment. In particular, the individuals who perform subjects' assessments and/or evaluate trial subject safety will be blinded.

For Substudy B, the investigator(s) will assign the responsibility of the unblinded dispenser/administrators to site personnel who will not participate in the evaluation of any trial subject. To ensure adequate coverage, at least two unblinded dispenser/administrators will be assigned per site. Site personnel or pharmacy personnel should fulfill these roles. Contact between the unblinded dispenser/administrator and trial subjects should be kept to a minimum.

The investigator, trial coordinator, and any site personnel other than the unblinded dispensers/administrators must not be allowed to know the trial treatment assigned to any trial subject and must not be allowed to see the trial treatment and any containers.

Trial subjects will be assigned to receive trial treatment according to the assigned vaccine dose group as summarized in Table 1. For further details, see the Sections 13.3 (Substudy A), 14.3 (Substudy B), and 15.3 (Substudy D).

The sponsor may decide to not activate one of the groups in Substudy B, in that case Substudy B will be a one group open-label substudy.

In the event of a Quality Assurance audit, the auditor(s) will be allowed access to unblinded trial treatment records at the site(s) to verify that randomization/dispensing has been done correctly.

6.6.2.3 Blinding of the sponsor and CRO personnel

To facilitate rapid review of data, sponsor and some CRO personnel will be unblinded to trial treatment allocation in this trial. However, CRO personnel 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.

6.6.2.4 Blinding of trial IRC members

In the substudies, to enable the trial IRC to provide real time medical oversight of trial subject safety during the conduct of this trial, the trial IRC members will include unblinded sponsor personnel.

6.6.2.5 Unblinding (breaking the blind)

In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a trial subject's vaccine dose 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 prior to unblinding a trial subject's vaccine assignment unless this could delay further management of the subject.

If the investigator is unavailable, if required to protect trial subject safety, an external physician may contact the trial medical monitor or trial site personnel to request unblinding of a subject's vaccine assignment.

If a trial subject's vaccine assignment is unblinded, the sponsor must be notified within 24 h 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).

6.7 Trial treatment compliance

Trial subjects will receive trial treatment directly from the responsible site personnel. The date and time of each dose administered must be recorded in the source documents and in the CRF. The IMP, the dose of IMP, the site of IMP administration, and the subject identification number will be confirmed at the time of dosing by the person administering the trial treatment (for further details, see the Pharmacy Manual).

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 concomitant therapies during the trial

The following medications are prohibited in this trial. Administration of such medications might lead to exclusion of the subject from analysis. See also the exclusion criteria listed in Section 5.4.

- Non-trial vaccines within 28 d before Dose 1 and until Visit 9 inclusive (Visit 11 for Substudy D; for exceptions, see Section 6.9.2).
- Any Orthopoxvirus-based vaccines, including vaccines for smallpox, vaccinia disease or mpox prevention, or vector Orthopoxvirus-based vaccines.
- Non-trial IMP.
- Blood/plasma products and/or immunoglobulins.
- Immunosuppressive medications including radiotherapy or systemic corticosteroids if administered at a dose of >20 mg/day of prednisone or equivalent for >14 days.
- Prophylactic antipyretics and other pain medication to prevent symptoms associated with trial treatment administration.

If a subject receives a prohibited therapy, further IMP administration will be discontinued but the trial subject should stay in the trial and complete all assessments planned per SoA. (Exception: For subjects who received prophylactic antipyretics or other pain medication to prevent symptoms associated with trial treatment administration for the previous dose, and if this medication has been stopped, further IMP administration will not be discontinued.)

6.9.2 Permitted concomitant therapies during the trial

The following concomitant therapies are allowed during participation in the trial if medically indicated.

- Antipyretics and other pain-relieving medication to treat symptoms after IMP administration. Standard therapeutic dose of acetaminophen (preferable, at doses of up to 4 g/d), or a non-steroidal anti-inflammatory drug (NSAID) if acetaminophen is contraindicated.
- NSAID or pain-relieving medication for ongoing conditions.
- Seasonal influenza vaccine is allowed if administered at least 14 d before or after any IMP administration.
- Medically indicated vaccines (e.g., rabies, tetanus).
- Inhaled, topical, or locally injected corticosteroids (e.g., intraarticular or intrabursal administration), or systemic corticosteroids if administered at a dose of ≤20 mg/day of prednisone or equivalent for ≤14 days.
- Allergy treatment with antigen injections is allowed if administered at least 14 d before or after any IMP administration.

Other concomitant medications may be permitted on a case by case basis by the investigator. The trial medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.9.3 Recording of concomitant therapy during the trial

For the period defined in the respective SoA (Sections 13.3.4 for Substudy A, 14.3.4 for Substudy B, and 15.3.4 for Substudy D), all concomitant medications 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 in the CRF, the recorded information must include:

- Reason for use
- Dates of administration including start and end dates
- Dose, frequency, and route of administration.

For further details, see the respective CRF completion guidance.

6.10 Treatment for specific adverse reactions

Expected adverse reactions are mostly mild to moderate local and systemic reactogenicity events. Additionally, anaphylaxis can occur after any vaccination. For further details see the BNT166 IB.

Treatment of these adverse reactions is at the discretion of the investigators; the following suggestions are provided:

- After the first occurrence of flu-like symptomatology including fever, subjects can be treated with standard therapeutic dose of acetaminophen (preferable, at doses of up to 4 g/d), or a NSAID if acetaminophen is contraindicated.
- Ensure adequate hydration of trial subjects on the day of IMP administration, e.g., by asking trial subjects to drink fluids (e.g., water) before the visit and during the visit per trial site standard.

If subjects experience no improvement of the symptoms after 3 d, or symptom kinetics are inconsistent with a relationship to IMP administration, additional diagnostic measures should be considered, and the trial medical monitor should be informed.

7 DISCONTINUATION OF TRIAL TREATMENT AND TRIAL SUBJECT DISCONTINUATION/WITHDRAWAL

In case of safety concerns it is investigator responsibility to decide on subject management and if trial treatment should be discontinued and/or if subject participation in the trial should be discontinued. The investigator should inform the sponsor about safety concerns as soon as possible upon awareness.

7.1 Permanent discontinuation or temporary pausing of trial treatment

The pausing rules for the groups and rules for the permanent discontinuation of individual subjects are provided in Sections 7.1.1 and 7.1.2.

The investigator must notify the sponsor as soon as possible but not later than 24 h after becoming aware about any AEs supporting pausing rules via the electronic data capture (EDC) system or paper SAE Form.

The trial medical monitor and safety physician will review the safety data on an ongoing basis, interacting with trial sites as needed, and following up on pausing rules.

For all cases, any trial IRC recommendations will be based on assessment of the totality of available data.

7.1.1 Group trial treatment pausing rules

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

Pausing rules criteria (only applicable to Phase I substudies) are the following:

- Any SAE developed by a subject dosed with trial treatment (at any dose level), which is assessed by the investigator, or the sponsor as related to IMP, or for which there is no alternative, plausible, attributable cause.
- Any case of fever >40.0°C/>104.0°F (measured orally) persisting for over 24 h despite the use of antipyretics after administration of trial treatment (at any dose level) that is assessed as related to IMP by the investigator, or for which there is no alternative, plausible, attributable cause.
- Grade 3 unsolicited AEs (including laboratory abnormalities), in two subjects in the same group, assessed as related to IMP by the investigator, and/or for which there is no alternative, plausible, attributable cause. Note: Local as well as systemic reactogenicity events such as injection site reactions and fever respectively (listed in Section 8.3.6) which occur within 7 d after IMP administration, independent of if they were reported in e-diary or as AE, are excluded from this pausing rule.
- Any Grade 3 or 4 hypersensitivity or any anaphylactic AE that occurs within 7 d of dosing with trial treatment for which there is no alternative, plausible, attributable cause.
- Regardless of the type, any scenario where Grade 3 solicited local reactions or systemic reactions confirmed by investigator or a medically qualified person is reported in three or more subjects in the same group, i.e., ≥20% of total 16 subjects in any group of Substudy A or in Substudy B.
- Any immune mediated event, such as myocarditis or pericarditis that is assessed as related to IMP by the investigator and for which there is no alternative, plausible, attributable cause.
- Any Grade 4 AEs including solicited adverse reactions assessed as related to the IMP (for cases of fever, see the above criteria related to fever for further clarification).

NOTE: Reactogenicity e-diary data confirmed by the investigator or a medically qualified person as being entered by the subject in error will not contribute toward a pausing rule.

In case the AE information received does not fully substantiate the pausing rule, and more information is required, the sponsor may still temporarily pause dosing in the affected groups and/or the same or higher dose levels or in the whole trial. In such cases the sponsor must promptly investigate the case.

If, upon investigation, the safety event that triggered the temporary pause of dosing is downgraded or no longer considered related to IMP by investigator (e.g., severe headache initially reported as related to IMP, but later considered not severe or not related), the pausing rule will be considered unconfirmed and dosing will be resumed.

If the sponsor confirms that any of the pausing rules criteria is met, the following actions will be undertaken:

- Assignment and trial treatment administration in the affected group and the same and higher dose levels, for all IMPs, including administration of Dose 2, will be paused.
- *Ad hoc* trial IRC meetings will be triggered, and the trial IRC will review all available data relevant to the issue at hand in an unblinded fashion.
- For all subjects who have already received IMP, all other routine trial conduct activities, including ongoing data entry, reporting of AEs, subject reactogenicity e-diary completion, blood sample collection, and follow-up, will continue.

After confirmation of pausing criteria being met, the trial IRC may recommend resuming treatment in a particular groups or dose levels, or permanent discontinuation of the trial treatment in a group or dose level, or overall. If required by the local regulatory authorities (e.g., by the Medicines and Healthcare products Regulatory Agency [MHRA]), a substantial amendment will be submitted when dosing is planned to be resumed after confirmed group trial treatment pausing criteria are met.

7.1.2 Permanent discontinuation of single trial subjects from trial treatment

It may be necessary to permanently discontinue a subject from the trial treatment. The following might be the reasons for such a treatment discontinuation: AEs including worsening of intercurrent illnesses, subject request, investigator request, protocol deviation.

The trial treatment of a single trial subject must be discontinued in the following cases:

- Suspected or confirmed immune-related AEs assessed as related to IMP (see Section 12.4 for myocarditis and pericarditis).
- Any Grade 4 local reaction or systemic event or unsolicited AE assessed as related to IMP.
- Any Grade 3 unsolicited AE assessed as related to IMP, except for the reactogenicity symptoms as listed in Section 8.3.6.
- Severe allergic AE (hypersensitivity or anaphylaxis) assessed as related to IMP.
- Any SAE assessed as related to IMP.
- Any trial subject pregnancy.

A permanent discontinuation of trial treatment for single subjects may also occur for non-medical reasons at the judgment of the investigator or the subject.

The trial IRC may recommend a permanent discontinuation of a single trial subject from trial treatment.

Trial subjects permanently discontinued from trial treatment will remain in the trial and will complete all assessments up to and including Visit 5 (Visit 6 for Substudy D) as listed in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4

[Substudy D]). At subsequent visits, trial subjects will complete the following assessments: AEs, MAAEs, AESIs, SAEs, pregnancies, and concomitant medication since last visit should be recorded, and subjects should be counseled/reminded to perform contraception, all as listed in the respective SoA. Other assessments can be performed if medically indicated. The investigator should make every effort to perform on-site visits or, if a trial subject is not able to visit the site, to collect the information via phone calls.

7.2 Trial subject discontinuation or withdrawal from the trial

A trial subject may be withdrawn from the trial at any time at his/her own request or may be discontinued from the trial at any time at the discretion of the investigator for behavioral, compliance, or administrative reasons.

If the trial subject withdraws consent, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

For trial subjects who withdraw consent, the investigator must clarify whether consent for research sample storage/processing (if given) is also withdrawn. If yes, then they must be informed that any research samples collected will be destroyed. The investigator must document research sample destruction in the ISF, inform the sponsor about the withdrawal of consent immediately, and record the withdrawal of consent in the EDC system.

If possible, permanently discontinued trial subjects should complete all assessments planned for the Early Termination Visit according to Section 8.11. If the reason for withdrawal from the trial is withdrawal of consent, then no additional assessments are allowed.

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 permanently discontinued trial subjects

Permanently discontinued trial subjects will not be replaced.

8 TRIAL ASSESSMENTS AND PROCEDURES

The investigator (or an appropriate delegate at the trial 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 protocol deviations, and are not allowed.

See the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]) for all planned timepoints 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. 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 activity as planned. 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 trial site prior to initiation of the trial.

Procedures conducted as part of the trial subject's routine clinical management and obtained before signing of the informed consent may be utilized for screening or baseline purposes provided the procedures meet the protocol-specified criteria and are performed within the timeframe defined in the respective SoA.

8.1 Collection of demographic and other baseline characteristics

At the visits listed in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]), the following data will be recorded for all trial subjects: age, sex, ethnic group, race, derived body mass index, and medical history information (including prior medication, smallpox vaccination status, and history of mpox, smallpox or vaccinia infections).

8.2 Efficacy assessments

Not applicable.

8.3 Safety assessments

Planned timepoints for all safety assessments are provided in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]). Unscheduled safety assessments may be performed at any time during the trial for any safety issues.

8.3.1 Physical examinations

Complete and symptom-orientated physical examinations will be performed at the timepoints listed in the SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

A complete physical examination will include, at a minimum, assessments of the skin, lymphatic nodes, cardiovascular, respiratory, gastrointestinal, and neurological systems.

A brief (symptom-directed) physical examination may be performed if deemed necessary by the investigator based on clinically relevant symptoms, signs, or medical history. A complete physical examination may be performed at any time if indicated in the investigator's opinion.

8.3.2 Vital signs, height, and body weight

Vital signs (comprising systolic/diastolic blood pressure, pulse rate, respiratory rate, and oral body temperature) will be assessed at the times given in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

Vital signs measurements 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). Vital signs will be measured with trial subjects in seated position.

Vital sign measurements should be performed before any planned blood collection.

Systolic/diastolic blood pressure, and pulse will be measured with a completely automated device. Manual techniques will be used only if an automated device is not available.

Height and body weight will be measured once at screening.

8.3.3 12-lead electrocardiograms

Standard 12-lead ECGs will be recorded at the times given in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF (corrected QT interval by Fridericia) intervals.

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 prior to blood collection, measurement of vital signs, and/or IMP administration.

All ECGs will be reviewed and evaluated by an investigator or cardiologist; the ECG report should include description of all abnormalities detected and assessment of their clinical significance. The heart rate, PR, QRS, QT, and corrected QT intervals and all abnormalities with indication of clinical significance will be recorded in the CRF.

If a clinically significant finding is identified (including, but not limited to changes from baseline in QT interval corrected according to Fridericia) at or after enrollment, the investigator may forward the ECG for evaluation by a cardiologist. This forwarding must be documented and the final ECG evaluation must be recorded in CRF.

Clinically significant abnormalities must be reported as AEs.

All original ECG readings must be kept as source documents.

8.3.4 Clinical laboratory tests

Blood sampling and urine collection for clinical laboratory tests will be performed at the times given in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]). The clinical hematology, chemistry, and urine analysis tests will be performed at a central laboratory.

The below listed clinical laboratory parameters will be assessed. Blood sampling and urine collection 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 (for subjects in screening, see chemistry and hematology re-test criteria in Section 5.4).

- Chemistry: alkaline phosphatase (ALP), alanine transaminase (ALT), creatinine, albumin, amylase, aspartate transaminase (AST), total bilirubin, blood urea nitrogen, glucose, lipase, sodium, potassium, calcium; troponin I at screening only. Estimated glomerular filtration rate (eGFR) will be calculated.
- Hematology: hemoglobin, hematocrit, red blood cell count, white blood cell count and differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelet count.
- Dipstick urine analysis: glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, and leukocytes.
- Microscopic urinalysis: if warranted by dipstick results, urine sediment will be microscopically examined for the presence of red blood cells, white blood cells, casts, crystals, epithelial cells, and bacteria.
- Only in volunteers born female where the postmenopausal status needs to be confirmed: Follicle stimulating hormone (FSH) at Visit 0.
- Only for VOCBP: serum beta human chorionic gonadotropin (hCG) at Visit 0 and urine test on the day of dosing before IMP administration.

All protocol-required clinical laboratory tests must be conducted in accordance with the central laboratory standard. The investigator must review the laboratory report, and document this review and assessment with their signature and date of signature.

All laboratory tests with values considered abnormal and clinically significant during participation in the trial (for subjects in screening, see chemistry and hematology re-test criteria in Section 5.4) should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or trial 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 should be notified. Abnormal clinically significant values should be documented as AE in the CRF.

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

Clinical laboratory test results will be categorized in accordance with the US FDA guidance for industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials".

8.3.5 Viral screening

Viral screens will be performed at the visit given in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]). The viral screen comprises a screen for human immunodeficiency virus (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) and reporting of antipyretic/analgesic medication

Subjects will complete e-diaries via an application installed on a provisioned device or on the subject's own personal device.

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 antipyretic/analgesic medication 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.
- Complete e-diary at approximately the same time every evening.
- Contact the site if they experience any severe or potentially life-threatening reactogenicity events.

The grading scales used in this trial to assess local reactions and systemic events are based on the US FDA guidelines on toxicity grading scales for healthy adult volunteers enrolled in preventive vaccine clinical trials (US FDA 2007). Reactogenicity events of Grade 3 and 4 will be confirmed by the investigator or a medically qualified person.

8.3.6.1 Assessments of intensity for local reactions

Pain (perceived) at the injection site will be assessed as absent, mild, moderate, severe, or potentially life-threatening, according to the grading scale in Table 2.

Subjects will be provided with a ruler to measure erythema / redness and induration / swelling. Erythema / redness and induration / swelling will be measured at the greatest single diameter and recorded and then categorized during analysis as absent, mild, moderate, severe, or potentially life-threatening, based on the grading scale in Table 2.

Table 2: 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.

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

8.3.6.2 Assessments of intensity for systemic reactions

Symptoms of systemic reactions will be assessed as absent, mild, moderate, severe, or potentially life-threatening, according to the grading scale in Table 3.

In order to record information on fever, a thermometer will be provided to the subjects with instructions on how to measure oral temperature.

Table 3: Systemic reaction grading scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3) [a]	Potentially life-threatening (Grade 4) [a]
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 routine daily activity	Emergency room visit or hospitalization for severe headache
Fatigue / tiredness	Does not interfere with activity	Some interference with activity	Prevents routine daily activity	Emergency room visit or hospitalization for severe fatigue
Myalgia / muscle pain	Does not interfere with activity	Some interference with activity	Prevents routine daily activity	Emergency room visit or hospitalization for severe muscle pain
Arthralgia / joint pain	Does not interfere with activity	Some interference with activity	Prevents routine daily activity	Emergency room visit or hospitalization for severe joint pain
Chills	Does not interfere with activity	Some interference with activity	Prevents routine daily activity	Emergency room visit or hospitalization for severe chills
Fever (oral temperature of ≥38.0°C/≥100.4°F)	38.0°C/100.4°F to 38.4°C/101.1°F	38.5°C/101.2°F to 38.9°C/102.0°F	39.0°C/102.1°F to 40.0°C/104.0°F	>40.0°C/>104.0°F

a. Investigator or medically qualified person confirmation is required.

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

8.3.7 Subject e-diaries for assessment of reactogenicity (local and systemic reactions) – Trial site personnel and investigator tasks

Subject e-diaries will be issued, trained, and collected by trial site personnel at the visits given in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

The trial site personnel will ask/remind the trial subjects to:

- Record the worst grade for each symptom in the e-diary at approximately the same time every evening on the day of IMP injection and then every day in the evening for a total of seven consecutive days.
- Measure their oral body temperature using the provided device and record their body temperature in the e-diary every day including the day of IMP injection.
- To contact the site immediately in case of severe or potentially life-threatening reactogenicity events.

Data on local reactions and systemic events reported in the e-diary will be transferred electronically to a third-party vendor, where they will be continually available for review by investigators and sponsor clinicians via an internet-based portal. Investigators will review subject-reported e-diary data daily for 7 d after each IMP dose. Investigators will discuss

their symptoms with the subjects, assess them and report in EDC any missed or not correctly reported events. Sites need to make every effort to contact subjects when the e-diary entry of the previous day was missed. If a Grade 3 or 4 reaction is reported in the e-diary, the trial site personnel must contact the subject (for example via a telephone contact) as soon as possible to ascertain further details and determine whether a site visit is clinically indicated.

If a trial subject experiences a potential Grade 3 or 4 reactogenicity, the subject will be asked to immediately notify the investigator or other medically qualified person, who will then confirm the grading. Only an investigator or medically qualified person is able to confirm a Grade 3 and Grade 4 event.

If a trial subject experiences confirmed Grade 3 or 4 reactogenicity, the investigator must notify the sponsor within 24 h after the grade of event was confirmed using the SAE Form and the EDC system.

If a trial subject experiences confirmed Grade 4 reactogenicity determined to be related to the IMP, further IMP administration to that subject will be discontinued (see Section 7.1).

The investigator must obtain the stop dates for those reactogenicity symptoms and antipyretic/analgesic medication used to treat symptoms associated with IMP administration that started on Days 1-7 and continued after the seventh day, document these stop dates in the source documents and in the CRF.

Reactogenicity symptoms (local reactions and systemic events) reported by the subjects in the e-diary should not be reported as AEs in the CRF, however the following should be reported as AE/SAE:

- Reactogenicity symptoms associated with IMP administration that started on Days 1-7 and continue after the seventh day;
- Reactogenicity symptoms associated with IMP administration which are SAEs or medically attended adverse events (MAAEs; for a definition, see Section 10.4.1).

8.3.8 Injection site reactogenicity assessments

Investigators will assess the injection site for reactogenicity symptoms at the visits and times given in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

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.

8.3.9 Subject monitoring after each IMP dose

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

8.3.10 Subject hotline

Trial subjects will be provided with contact details for a 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.

8.3.11 Wellbeing phone calls

Subjects will be contacted by phone by medically qualified staff for non-leading wellbeing calls at the times given in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

8.4 Adverse events and SAEs

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

8.4.1 Time period and frequency for collecting AE, MAAE, adverse events of special interest (AESI), and SAE information

All AE and SAE information will be collected at the timepoints specified in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]):

- AE (including MAAE) collection will start once informed consent is given and will continue until Visit 9 (Visit 11 for Substudy D).
- AESI and SAE collection will start once informed consent is given and will continue until Visit 12 (Visit 14 for Substudy D) or the end of subject participation in the trial.

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 to not introduce bias when detecting AEs and/or SAEs.

Guidance for the recording, evaluating, and assessing of AEs and SAEs is provided in Section 10.4. The US FDA guidance (US FDA 2007) will be used to grade the severity of AEs and SAEs.

Investigators are not obligated to actively seek AEs or SAEs 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 the investigator considers the event to be related to the trial treatment or trial participation (see Section 10.4, for guidance on the assessment of causality), the investigator must promptly notify the sponsor as described in Section 10.4.4.

The sponsor may request that the investigator obtain specific follow-up information in an expedited fashion.

Prompt notification of SAEs and events which may contribute to the pausing rules 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 events which may contribute to pausing rules criteria, all such events will be recorded and reported to the sponsor or designee within 24 h after the site becomes aware of the event using the SAE Form and the EDC system.

All SAEs (initial and follow-up reports) will be recorded and reported to the sponsor or designee within 24 h after the site becomes aware of the event using SAE Form, as indicated in Section 10.4.4.

For the reporting of AESI, see Section 8.4.8.

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, or the trial subject withdraws consent (as defined in Section 7.3). If no final status is reached by Visit 12 (at ~1 year after Dose 2; Visit 14 for Substudy D), the investigator must confirm the unavailability of a final status. Further information on follow-up procedures is provided in Section 10.4.3.

For subjects who are screen failures, the AE 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.

New or updated information will be recorded in the originally completed CRF.

If a trial subject dies during participation in the trial the investigator will provide to the sponsor a copy of any postmortem findings including histopathology if available.

All SAEs follow-up reports will be recorded and reported 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 suspected unexpected serious adverse reactions (SUSARs)

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 (IRBs)/Independent Ethics Committees (IECs), and investigators. The execution of reporting to the different entities may be delegated as detailed in the trial-specific Safety Management Plan.

Investigators will inform the local IEC/IRB where applicable.

All serious adverse reactions, the nature, severity or outcome of which is not consistent with the reference safety information are “unexpected serious adverse reactions”. The expectedness assessment for all related SAEs is based on the reference safety information included in Section 6.1.5 of the BNT166 IB.

All suspected adverse reactions related to an IMP that occur in this trial, and that are both unexpected and serious qualify as SUSARs. SUSARs are subject to expedited reporting requirements according to applicable regulatory requirements and guidance (e.g., International Council for Harmonisation [ICH] E2A guidance). Information about analysis of similar events will be added to the SUSAR report.

As events of myocarditis and pericarditis are considered AESI (see Section 8.4.8), all temporally related reports of myocarditis and pericarditis will be eligible for expedited reporting in the same fashion as SUSARs regardless of meeting any seriousness criteria. For guidance on the management of symptoms that could represent myocarditis or pericarditis, see Section 12.4.

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

8.4.5 Pregnancy testing and reporting of pregnancies

For VOCBP, pregnancy tests and counseling will be performed at the times given in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

Pregnancy information will be collected over the time period defined in respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]). For guidance on the collection of pregnancy information, see Section 10.5.3.

Any trial subject who becomes pregnant while participating in the trial will need to discontinue the IMP immediately (see Section 7.1.2).

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 Section 10.4.2.2. 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 one 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.

8.4.7 Disease-related events/outcomes not qualifying as AEs or SAEs

Not applicable.

8.4.8 Adverse events of special interest

An AESI, serious or non-serious, is one of scientific and medical concern specific to the sponsor’s product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor are appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the trial sponsor to other parties (e.g., regulators) might also be warranted.

Any events of (suspected) myocarditis and pericarditis, will be reported on an expedited basis as AESI regardless of associated seriousness criteria. AESI should be reported within 24 h of site awareness using the AESI Report Form and the contact details given in Section 10.4.4.

For guidance on the management of symptoms that could represent myocarditis or pericarditis, see Section 12.4.

All AESIs will be followed up until resolution, stabilization, or the event is otherwise explained, or until the trial subject is lost to follow-up (as defined in Section 7.3).

Each event of myocarditis and pericarditis will be assessed for the level of diagnostic certainty as per the established standard case definition from the Brighton Collaboration by the IRC.

8.5 Pharmacokinetics

Pharmacokinetic parameters are not evaluated in this trial.

8.6 Genetics

Genetic parameters are not evaluated in Substudies A and B.

For Substudy D, the planned genetic analyses comprise different approaches to further elucidate immune responses to the BNT166a vaccine as detailed in Section 8.8, e.g., analysis of T cell and B cell receptor repertoire by next generation sequencing and/or single-cell RNA sequencing, transcriptomic analysis, and human leukocyte antigen (HLA) typing.

Genetic analyses are part of Substudy D and will be reflected in the ICF as such. A subject’s consent and consent withdrawal will be recorded in the CRF. Sampling will be performed at the timepoints listed in the SoA in Section 15.3.4.

All data generated using the genetic samples will be handled in accordance with applicable laws and regulations; this includes requirements applicable for data protection, sample shipment, and a potential withdrawal of consent.

8.7 Immune responses

Immune responses will be assessed at the times listed in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

Instructions on the sample collection, handling, and shipping will be provided in a Laboratory Manual.

Immune response analyses are categorized as humoral immune responses, cell-mediated immune responses, or innate immune responses. Among the different assays, priority will be given to those that are considered to be most actionable in terms of decision making during further clinical development of the BNT166 multivalent vaccine candidates.

Humoral immune response assessments will include:

- Levels of vaccinia virus specific neutralizing antibodies using (e.g.) plaque reduction neutralization test (PRNT).
- Levels of MPXV-specific neutralizing antibodies using (e.g.) PRNT or microneutralization assays.
- Levels of BNT166 multivalent vaccine candidate antigen-specific binding antibodies using (e.g.) enzyme-linked immunosorbent assay (ELISA) or similar assay.

8.8 Additional research analyses

In Substudy D, samples will be collected specifically for additional research analyses per the SoA (Section 15.3.4). Planned additional research analyses will include the evaluation of antigen-specific cluster of differentiation (CD) 4 and CD8 T cells, and the levels of circulating cytokines. A systems serology approach will be used to more comprehensively characterize the humoral response to the vaccine, including measurement of antibody isotype/subclass, affinity, and functional antibody-mediated responses. Further, transcriptomic analysis will be used to characterize the molecular and cellular networks that influence innate and adaptive immune responses to BNT166a.

In selected subjects, future additional research analyses will be conducted using residual biological samples. For all substudies, future additional research analyses to be performed on residual samples may include, but are not limited to: phenotypic or functional characterization of antigen-specific T cells or B cells (e.g., by flow cytometry-based phenotyping including multimer staining); analysis of T cell and B cell receptor repertoire (e.g., by next generation sequencing and/or single-cell RNA sequencing); and evaluation of biophysical and functional properties of antigen-specific antibodies. Phenotypic or functional characterization of other immune cell populations that may be relevant to understanding the vaccine-induced immune responses may be included in this research. Residual samples may be used to inform the development of other vaccines or vaccine-related products, and/or for vaccine-related assay work supporting vaccine programs or further scientific research purposes.

Biosamples for these additional analyses will 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 biosample will be labeled with a number (optionally also with a bar code) and will not include information that could be used to identify the subject. Results of the analyses will be linked to the clinical information collected during the trial using this specific number. Additional research biosamples and all data generated using the biosamples will be handled in accordance with applicable laws and regulations; this includes requirements applicable for data protection and a potential withdrawal of consent. A subject may request that their samples, if still identifiable, be destroyed at any time, however, any data already collected from these samples will still be used for research. The biological samples may be shared with other research organizations as long as confidentiality is maintained, and no testing of the subject's DNA is performed.

For blood draw timepoints and volumes, please refer to Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], 15.3.4 [Substudy D], and Section 8.9.

8.9 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 in case of AEs or SAEs.

8.10 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 (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

8.11 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. If possible, all assessments planned for the actual week or day of that visit as listed in respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]) and which are not included in the Early Termination Visit should also be performed.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical hypotheses

No formal statistical hypotheses will be tested in this trial.

9.2 Interim analyses and analysis sequence

Interim analyses of Substudies A and B 1 month post-Dose 2 safety, reactogenicity, and immunogenicity data will be performed to inform further clinical development and to select the dose for Substudy D. An interim analysis of Substudy D 1 month post-Dose 2 safety,

reactogenicity, and immunogenicity data will also be performed to inform further clinical development. Interim analyses may also be performed at other timepoints.

A final analysis will be performed once all subjects have completed the last planned visit.

9.3 Sample size determination

No formal sample size calculations have been performed. The sample size for each group is mainly driven by typical FIH Phase I designs for the 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 an IMP. The probability of observing at least one AE for a given true event rate of 10% is 81.5% in 16 subjects per group in Substudies A and B. For Substudy D, the probability of observing at least one AE for a given true event rate of 10% is 96.6% in 32 subjects. The sample size of 32 subjects in Substudy D has been chosen to provide supporting information regarding the immunogenicity of the IMP.

9.4 Analysis sets

The following analyses sets are defined:

Analysis set	Description
Screened Set	All subjects who provided informed consent.
Safety Analysis Set	All subjects who received at least one dose of the IMP.
Immunogenicity Analysis Set 1	All subjects who • received at least one dose of IMP, • had a valid immune response assessment within an appropriate window, • had no important protocol deviations that can confound immunogenicity data as determined by the sponsor.
Immunogenicity Analysis Set 2	All subjects who • received two doses of IMP, • had a valid immune response assessment within an appropriate window, • had no important protocol deviations that can confound immunogenicity data as determined by the sponsor.

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 statistical analysis plan (SAP) will be finalized before the first database lock, 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 identified and justified in the SAP and reported in the clinical study report.

This section gives a summary of the planned statistical analyses of the most important endpoints including primary endpoints.

9.5.1 General considerations

No formal statistical testing will be done. In general, descriptive summary tables and figures will be presented for each substudy by dose level and multivalent vaccine candidate. Where appropriate, data will also be summarized overall across substudies per multivalent vaccine candidate.

Continuous variables will be summarized by group using the following descriptive statistics: number of subjects (n), mean, standard deviation, median, minimum and maximum, unless otherwise stated.

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

Categorical variables will be summarized by group presenting absolute and relative frequencies (n and %) of subjects in each category. Baseline is defined as last available value prior to first IMP administration. Where appropriate, changes from baseline (pre-Dose 1) will be assessed.

9.5.2 Safety analyses including the primary endpoints

For the safety endpoints, see the respective substudy sections (Sections 13.3 [Substudy A], 14.3 [Substudy B], and 15.3 [Substudy D]).

All safety analyses in Substudies A, B, and D will be based on the Safety Set.

All AEs will be coded to a system organ class and preferred term using the most recent version of the MedDRA coding system.

Reactogenicity – subject-reported via the e-diary

Descriptive statistics will be provided for each reactogenicity endpoint for each dose and by dose level and vaccine candidate. In general, solicited reactions will be analyzed for each IMP administration, i.e.:

- Up to 7 d after each IMP dose

For each IMP administration, the number and proportion (%) of subjects reporting at least one local reaction at the injection site or systemic event will be summarized for each of the following types:

- Any local reactions or systemic events
- Local reactions or systemic events by severity

For each IMP administration, the local reactions will be summarized by worst grade for each reaction. Systemic events will be summarized in the same manner as for local reactions.

Concomitant medications administered due to local reaction and systemic events during the 7 d (full days) after each IMP dose will be summarized.

Bar charts with the number and percentage of subjects with local and systemic events will be provided.

Additional safety analyses may be described in the SAP.

Unsolicited AEs (for a definition of unsolicited, see Section 10.7.3)
Descriptive statistics with number and proportion (%) of subjects reporting at least one AE (defined in Section 10.4.1) in the following categories will be summarized for each of the following AE types for the intervals from Dose 1 to 28 d after Dose 1 and from Dose 2 to 28 d after Dose 2:

- Any AE
- Related AE
- Grade of AE
- Related Grade ≥3 AE
- Any solicited AE that continues longer than 7 d post-IMP administration
- AEs leading to permanent discontinuation of trial treatment
- MAAEs; for a definition, see Section 10.4.1
- Related MAAE
- AESI; for a definition, see Section 8.4.8
- Related AESI

Descriptive statistics with number and proportion (%) of subjects reporting at least one SAE (defined in Section 10.4.2) starting from the first dose until Visit 12 (24 weeks after Dose 2; Visit 14 for Substudy D) will be summarized for each of the following SAE types:

- Solicited AE which are SAE
- Any SAE
- Related SAE
- Any death

Moreover, the number and proportion (%) of subjects with AEs from the first dose will be summarized by the preferred term nested within system organ class for all AEs, SAEs, IMP-related AEs, AEs leading to discontinuation, and AEs by severity as appropriate.

Additional safety analyses may be described in the SAP.

9.5.3 Immunogenicity analyses defined as secondary and exploratory endpoints

Secondary and exploratory endpoints are defined in Sections 13.4 (Substudy A), 14.4 (Substudy B), and 15.4 (Substudy D). They will be analyzed by summary statistics within the Immunogenicity Analysis Sets specified in Section 9.4.

Details of these analyses will be described in the SAP.

9.5.4 Other analyses

Details of the other analyses 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.

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, EU Regulation No 536/2014 (if applicable), and all other applicable local regulations.

- Informing the sponsor immediately of 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 Recruitment and the informed consent process

The investigator and trial site staff at each trial site will oversee all trial recruitment and enrollment activities. Trial subjects will be recruited by local advertising, community engagement methods, and/or internal database typically used by the trial site to target the trial population. For a detailed description of the recruitment procedures, see the trial recruitment plan.

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.

10.1.4 Data protection

All data collection 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 their 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.

Even if a subject withdraws from the trial or is withdrawn from the trial, the trial site may contact the subject for follow-up activities, for example to obtain some more information about their health if they were experiencing a health problem at the time they withdrew from the trial. This information may be shared with the sponsor and regulatory authorities to fulfil legal obligations and for reasons of public interest in the area of public health, in particular to ensure high standards of quality and safety in healthcare and medicines.

10.1.5 Committees – IRC

A trial IRC will be established to provide medical oversight of trial subject safety during the conduct of this trial, with a focus on guidance, management of emergent safety issues, to monitor and implement safety with respect to pausing rules, to recommend a pause, stop or restart of the trial treatment at the individual level, group level or at the trial level and overall decision making as outlined in the trial IRC Charter. It includes periodic in-depth review of safety data by trial subject, group, and cumulatively, in order to confirm mechanism of action, identify potential off-target toxicities, and to understand the IMP's

safety profile and feasibility for further clinical development. The trial IRC is also a forum for the discussion of other data which could impact the IMP benefit-risk assessment, thereby allowing the trial IRC to assess the overall benefit-risk of the IMP.

The trial IRC is comprised of BioNTech clinical and safety physicians as voting members and department representatives for safety management, program management, data management, biostatistics, and clinical operations, as well as external representatives as non-voting members. The trial IRC will be constituted and act according to procedures described in the trial IRC Charter. The trial IRC will prepare written minutes of its meetings.

Ad hoc IRC meetings may be triggered by AEs or any other topic that could compromise the safety of the subjects included in the trial (see Section 7.1.1).

All investigators will be informed about decisions linked to dose-escalation and progression to the further substudies in an expedited manner.

10.1.6 Dissemination of clinical trial data

A final ICH E3 conform clinical study report (CSR) integrating all trial results will be prepared by the sponsor.

The outcomes of additional research analyses will be reported according to the sponsor SOPs, i.e., independently of the clinical trial data.

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 EU Regulation No 536/2014, EU Regulation 1049/2001, and the US Final Rule, which implements Section 801 of the US Food and Drug Administration Amendments Act (FDAAA 801). Clinical documents under such laws includes protocols and protocol amendments, SAPs, and CSRs.

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 European Medicines Agency (EMA) website. Clinical data, under Phase I of this policy, includes clinical overviews, clinical summaries, CSR, and appendixes containing the protocol and protocol amendments, sample CRFs, and statistical methods. Under Phase II 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 outcome measures (irrespective of outcome) and lay summaries, will be posted on a publicly accessible website.

BioNTech may provide researchers secure access to subject level data, expert summaries, lay summaries, and CSRs for the purposes of “*bona fide* scientific research” that contributes to the scientific understanding of the disease, target, or compound class.

In these cases, all trial subject level data will be anonymized in accordance with applicable privacy laws and regulations.

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 10.1.9).

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., a 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 in accordance with relevant local requirements after trial completion unless 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 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, an investigator will be designated to coordinate the publication by mutual agreement.

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

10.1.10 Protocol preparation and approval

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

10.1.11 Investigators and trial administrative structure

10.1.11.1 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.

10.1.11.2 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 their 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.

10.1.11.3 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.

10.1.11.4 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.

Some sponsor tasks may be delegated, e.g., to CRO personnel. Documentation of any delegation of responsibilities will be maintained.

10.1.12 Premature termination or suspension of the trial

If the trial is prematurely terminated or suspended 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 or suspends 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 or suspension.
- If the sponsor terminates or suspends 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 or suspension.
- If the IRB/IEC terminates or suspends 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 or suspension.

10.1.13 Reporting of serious breaches

A serious breach is any deviation of the approved protocol version that is likely to affect to a significant degree the safety of a trial subject and/or the rights of trial subjects and/or the reliability and robustness of the data generated in the clinical trial.

Trial sites, including pharmacists, CROs and other designees of the sponsor, must report any suspected serious breaches promptly to the sponsor. Suspected serious breaches, as well as any communication related to suspected serious breaches, must be reported to the sponsor by e-mail using the following e-mail address within 24 hours of knowledge:

The sponsor will assess each suspected serious breach to decide whether it should be reported as a serious breach. The sponsor will comply with country-specific regulatory requirements relating to the reporting of serious breaches to regulatory authorities and IRBs/IECs.

10.2 Data collection and management

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

10.2.1 Data management

A 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 data entry into the EDC system. 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.

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 their 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 CRF completion guidance. The CRFs will be handled in accordance with instructions and be submitted electronically to the sponsor via the system.

All CRFs should be completed 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.

10.2.4 Investigator's Site File and the TMF

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 (additional information)

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

10.4 AEs: 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 trial subjects received medical attention defined as unscheduled hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including visits to emergency rooms or other medical facilities.
- AESI; for a definition, see Section 8.4.8.

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

- Any abnormal 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.
- 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 which are associated with the underlying disease or a pre-existing condition, 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).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the trial that do not worsen.

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 signs 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 level to which the subject was assigned (for BNT166a and BNT166c) 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

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

- results in death, or
- 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 tumors, 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 (rated as mild, moderate, severe or potentially life-threatening, as described in the US FDA guidance [US FDA 2007]); see Section 10.4.2.2 for guidance on SAE exemptions and Section 10.4.3 for guidance on the assessment of severity.

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 h of first knowledge 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 Regulation No 536/2014, 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 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.
- 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 caused by an acute worsening of the pre-existing condition during the time of 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.
- Routine treatment or monitoring of an underlying disease not associated with any deterioration in the trial subject's condition.

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. 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
    - 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.
- All AEs will be followed up until resolution, stabilization, until the event is otherwise explained, or until the trial subject is lost to follow-up (as defined in Section 7.3).

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 trial medical monitor should be consulted.

The intensity of (serious) AEs will be graded by the investigator. For further guidance refer to the US FDA guidance (US FDA 2007). Where specific guidance for an AE 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 extent 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); i.e.:
    - 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 on the site of injection of the trial treatment (e.g., redness, swelling, hematoma or itching) or during or after trial-specific procedure (e.g., discomfort after blood drawing). These events must be reported in the CRF on AE pages as “related to trial procedure” with the causing procedure specified.

Notes applicable for relationship to trial procedures including trial treatments

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 their 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

All SAEs (defined in Section 10.4.2) 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.:
- Safety Report E-Mail address:

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

Information for the final description and evaluation of a case report may not be available within the required timeframes 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 or the Additional Information and Follow-Up Form) without delay as described above and accompanied by appropriate pseudonymized 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 date 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 ISF.

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 trial 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

Volunteer 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.

Women in the following categories are not considered VOCBP:

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

  Note: Documentation for any of the above categories can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview. The method of documentation should be recorded in the subject's medical record for the trial.

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 female

For the purpose of this document, a postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

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 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 or ligation. [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

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

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 first knowledge 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 child at least two times (i.e., at the time of expected date of delivery and around the child's first birthday), and forward the information to the sponsor. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, 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.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication (spontaneous abortion, bleeding, preeclampsia etc.) 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 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.

10.5.4 Management of contraception

At timepoints indicated in the SoAs (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]), the investigator or designee will inform the trial subject of the need to use the prescribed contraception consistently and correctly, and will document the conversation. This will include advice about donation and cryopreservation of 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 outcome(s) at the timepoints indicated in the respective SoA (Sections 13.3.4 [Substudy A], 14.3.4 [Substudy B], and 15.3.4 [Substudy D]).

10.6 Liver and kidney safety: Suggested actions and follow-up assessments

10.6.1 Potential cases of drug induced liver injury

Drug liver injury (DILI) is an uncommon but potentially lethal adverse drug reaction. Drugs cause liver injuries by many different mechanisms (DILI 2020). These injuries resemble almost all known liver diseases and there are no pathognomonic findings, even upon liver biopsy, that make diagnosis of DILI certain. Therefore, when possible DILI is suspected, it is essential to ensure immediate management of the subject and to gather additional clinical and laboratory information necessary for differential diagnosis of the cause (for guidance, see the US FDA guidance on DILI 2009). In many cases, there is a delay of at least a few weeks between initiation of treatment and onset of liver injury. However, for some drugs, rapid onset of injury may occur, sometimes in the presence of a systemic hypersensitivity reaction that can be associated with multi-organ involvement, fever, eosinophilia, and/or rash cause (US FDA Guidance on DILI 2009). The algorithm below contains the minimum requirements for management of suspected DILI.

> If ALT and/or AST are >5 upper limit of normal (ULN) or if ALT and /or AST are >3 ULN AND total bilirubin >2 ULN or international normalized ratio >1.5 ULN, the subject should undergo a close observation, including physical examination. The subject must be questioned about symptoms potentially suggesting liver injury including anorexia, nausea, fatigue, right upper abdominal discomfort, and vomiting. The subject should also be questioned about other symptoms and relevant medical history including, but not limited to, concurrent diseases, alcohol consumption, acetaminophen/paracetamol (either by itself or as a co-formulated product), other prescription or over the counter medications, supplements, herbal medications, special diets, recreational drugs, surgeries and other medical interventions, family history, occupational/environmental exposure to chemicals etc.

The following laboratory parameters must be tested next within 48 to 72 h: ALT, AST, ALP, and total bilirubin. If the subject cannot come to the site for visit within the indicated timeframe, the tests should be done locally, and results and local normal ranges should be requested from the primary physician/point of care. The administration of a potential second dose (in accordance with the protocol) should be reconsidered by the investigator based on the subject's work-up results.

If the symptoms persist or laboratory abnormalities persist upon repeated testing, then, as a minimum, the following tests and assessments must be performed, close observation should be continued, and the site's standard for DILI management should also be followed, whatever is stricter. If the laboratory abnormalities remain of the same severity grade or become more severe, and/or the subject's condition deteriorates, and the investigator considers the case a potential DILI, the following should be performed:

- Laboratory tests: ALT, AST, ALP, direct and indirect bilirubin, creatine kinase, gamma glutamyl transferase, prothrombin time/international normalized ratio, albumin, creatinine, complete blood count. Liver function test (ALT, AST, ALP, total bilirubin) monitoring as closely as possible (every 48 h).
- Clotted blood sample and anticoagulated blood samples should be taken for further potential analyses to determine etiology.
- Physical examination.
- Rule out acute viral hepatitis types A, B, C, D, and E; recent infection with Epstein-Barr virus; herpes; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease.
- Consider liver imaging, e.g., ultrasound examination.
- Consider consulting a hepatologist.

A potential DILI (Hy's law) case becomes a confirmed case only after all results of reasonable investigations have been received and have excluded an alternative etiology. When confirmed, the case should be reported as a DILI SAE.

10.6.2 Laboratory assessment of change in kidney function and detection of kidney injury

Serum creatinine value will be monitored during the trial. An increase in serum creatinine of ≥0.3 mg/dL (or ≥26.5 µmol/L) or ≥1.5 times relative to the subject's baseline value should trigger another assessment of serum creatinine as soon as practically feasible, preferably within 48 h from awareness.

If the second assessment confirms elevated serum creatinine, the subject should return to the investigator site and be evaluated as soon as possible, preferably within 48 h from knowledge of the second assessment confirming abnormal serum creatinine result. This evaluation should include laboratory tests (serum blood urea nitrogen [BUN], serum creatine kinase, and serum electrolytes [including at a minimum potassium, sodium, phosphate/phosphorus, and calcium]), complete blood count, urinalysis including microscopic examination. Detailed medical history should be collected, and physical examination performed. All cases confirmed on repeat testing as meeting the laboratory criteria for acute kidney injury, with no other cause(s) of laboratory abnormalities identified, should be considered potential cases of drug-induced kidney injury irrespective of availability of all the results of the investigations performed to determine etiology of the abnormal serum creatine.

Diagnostic criteria for kidney injury (El-Khoury et al. 2021):

- Increase in serum creatinine by ≥0.3 mg/dL (26.5 µmol/L) within 48 h; or
- Increase in serum creatinine to ≥1.5 times baseline, known or presumed to have occurred in the past 7 d; or
- Urine volume <0.5 mL/kg/h for 6 h.

10.7 Standard definitions

10.7.1 Trial site start/closure and trial discontinuation

The date of first site open (first act of recruitment of potential trial subjects for this trial) is the “trial start date”.

The date when all trial sites are closed is the “date of trial closure”.

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 may 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 suspend the whole trial may include but are not limited to:

- When there are safety concerns (e.g., if there is a trial IRC recommendation).

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

- When there are safety concerns (e.g., if there is a trial 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. The investigator shall promptly inform the trial subjects and should assure appropriate follow-up.

10.7.2 Definition of enrolled subject

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

10.7.3 Definition of solicited and unsolicited safety data

All reactogenicity events information recorded via the subject e-diaries or solicited by the investigator are solicited events. All AE information or safety data that are voluntarily

communicated by the subject or collected by the investigator (ECG or laboratory results etc.) are considered unsolicited events.

10.7.4 Definition of trial completer

A trial subject is considered to have completed the trial if they have completed the last scheduled procedure shown in the respective SoA (Sections 13.3.4 for Substudy A, 14.3.4 for Substudy B, and 15.3.4 [Substudy D]).

10.8 Protocol amendments and updates

10.8.1 Protocol update from version 1.0 to 2.0

Protocol version 1.0 was updated to version 2.0 to reflect updates triggered by MHRA remarks dated 23 JUN 2023.

A comparison of every new sponsor approved protocol version with the next approved version is filed together with the protocol in the TMF.

See the table for a summary of the reasons for major changes compared to the previous version. Minor editorial changes, such as the correction of typing errors, are not specifically listed.

Section	Change made	Reason for change
6.9.1 Prohibited concomitant therapies during the trial	Text was updated to say that if a subject receives a prohibited therapy, further IMP administration will be discontinued.	MHRA request
6.9.2 Permitted concomitant therapies during the trial	Text allowing pre-existing stable disease was deleted.	MHRA request
7 Discontinuation of trial treatment and trial subject discontinuation/withdrawal	Text was updated to clarify that in case of safety concerns it is investigator responsibility to decide on subject management and if trial treatment should be discontinued and/or if subject participation in the trial should be discontinued.	MHRA request
7.1.1 Group trial treatment pausing rules	Text was updated to say that the pausing rule for Grade 3 solicited local and systemic reactions is regardless of type of reaction and to say that the threshold for group trial treatment pausing is 20% for all substudies.	MHRA request
13.5.3 Inclusion criteria	Text allowing treatment of pre-existing stable disease was deleted throughout the protocol.	MHRA request
14.5.3 Inclusion criteria	Text allowing treatment of pre-existing stable disease was deleted throughout the protocol.	MHRA request
15.5.3 Inclusion criteria	Text allowing treatment of pre-existing stable disease was deleted throughout the protocol.	MHRA request

10.8.2 Protocol update from version 2.0 to 3.0

Protocol version 2.0 was updated to version 3.0 to address FDA feedback given to the IND Module 1.11.3. clinical information amendment.

A comparison of every new sponsor approved protocol version with the next approved version is filed together with the protocol in the TMF.

See the table below for a summary of the reasons for major changes compared to the previous version.

Section	Change made	Reason for change
All pages	The date and document version number on all pages was updated to reflect this update.	This update
Title page and Section 1.1 Synopsis	Addition of the IND number.	This update
7.1.1 Group trial treatment pausing rules	The pausing rule “Any Grade4 AEs including solicited adverse reactions assessed as related to the IMP (for cases of fever, see the above criteria related to fever for further clarification)” was added.	FDA feedback
10.8.2 Protocol update from version 2.0 to 3.0	Section inserted to describe the rationale for this update.	This update

10.8.3 Protocol update from version 3.0 to 4.0

Overall update rationale

The update was made to introduce Substudy D. This substudy will further investigate and characterize immunogenicity of the investigational RNA-based multivalent vaccine candidate BNT166a by assessing not only binding and neutralizing antibodies, but also functional antibody responses, cell-mediated immunity, and innate immunity.

Other notable changes include the removal of the multivalent vaccine candidate BNT166c from Substudy C, the removal of the staggered dosing approach from the Substudy C design, and the addition of AESIs as an endpoint to the safety objectives for Substudies A, B, and C.

Description of changes

Minor editorial changes, such as the correction of typing errors or improvement of language for consistency, are not specifically listed. A comparison of every new sponsor approved protocol version with the next approved version is filed together with the protocol in the TMF.

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

Section	Reason for change
Multiple	Updated to reflect major changes in other sections.
Title page, 1.1	Added ClinicalTrials.gov identifier.
1.1, 2.1	Updated mpox information from WHO.
1.1, 13.3, 14.3	Sentence added to say that Groups A3 and B2 (planned to be dosed with BNT166c) will not be activated.
1.1, 4.1.2	Correction of trial duration.
2.2	Restructuring of text for consistency with IB; update and addition of mpox case numbers.

Section	Reason for change
2.3.2	Sentence added to refer to Section 5.3.1 of the BNT166 IB for a detailed risk assessment of the BNT166-encoded antigens.
2.3.3	Deletion of erroneous clinical chemistry and hematology test timepoint.
6.2	Updated to reflect data from the updated BNT162 IB.
6.9.1	Addition of an exception to the prohibited concomitant therapies: Further IMP administration will not be discontinued for subjects who received prophylactic antipyretics and other pain medication to prevent symptoms associated with trial treatment administration.
6.9.1, 6.9.2	Moved bullet point “allergy treatment” from prohibited concomitant therapies to permitted concomitant therapies to clarify timeframe for antigen injection allergy treatment.
7.1.2	Change regarding subjects permanently discontinued from trial treatment: Such subjects will only complete certain safety assessments post-Visit 5 (post-Visit 6 for Substudy D). Also, the investigator should make every effort to perform on-site visits or, if a trial subject is not able to visit the site, to collect the information via phone calls.
7.2	Clarification: The withdrawal of consent must also be recorded in the EDC system.
8.3.2	Correction: Respiratory rate will not be measured with a completely automated device.
8.3.4	Updated for clarification and to ensure consistency with exclusion criteria. Addition of urine analysis tests to the list of tests performed at a central laboratory.
8.3.7	Clarification: Confirmed Grade 3 or 4 reactogenicity events must also be reported via the EDC system.
8.4.2	Clarification: Events which may contribute to pausing rules criteria must also be reported via the EDC system.
8.6	Addition of planned genetic analyses for Substudy D.
8.7	Addition of exploratory assessments for Substudy D.
8.8	Updated to include additional research analyses for Substudy D (based on specifically collected samples) and for all other substudies (based on residual samples).
9.2	Updated to include dose selection process for Substudies C and D.
9.4	Clarification of Immunogenicity Analysis Set 1, addition of Immunogenicity Analysis Set 2.
9.5.3, 1.1	Text shortened, details on the immunogenicity analyses will be provided in the SAP.
10.1.3	Addition of subject recruitment information.
10.1.5	Addition of a description of the IRC members per previous competent authority request. Clarification of the triggers for *ad hoc* IRC meetings.
10.1.13	Introduction of language on reporting of serious breaches.
10.4.1.3.3	Clarification of overdose.
10.4.2	Clarification of “other situations”.
10.4.3	Addition of bullet point regarding AE follow-up.
10.5.1	Clarification of postmenopausal female definition.
10.6.1	Updated to give more detailed guidance on DILIs.
10.7.3	Updated for clarification.
13.4, 14.4, 15.4	Clarifications in reactogenicity and safety outcome measures regarding analysis time periods for local reactions, systemic events, AEs, SAEs, and AESIs to account for subjects who do not receive Dose 2.
13.4, 14.4, 15.4	AESIs added as endpoint to safety objectives per competent authority request.

Section	Reason for change
13.4, 14.4, 15.4	Deletion of the following safety outcome measure (and corresponding endpoint): "Proportion (%) of subjects with worsening grading shifts in hematology and chemistry laboratory values between baseline and 1 week after Dose 1, before Dose 2, 1 week and 1 month after Dose 2." As defined in this protocol, abnormal clinically significant values will be documented as AEs in the CRF. Any (serious) AEs resulting from laboratory test values will be graded by the investigator. Therefore, summarizing shifts in laboratory parameters might not add additional value as they do not include an investigator assessment and might not be clinically relevant.
13.4, 14.4, 15.4	Correction: "Seroconversion" changed to "seroresponse".
13.5.3, 14.5.3, 15.5.3	Inclusion criterion #9 updated for clarification.
13.5.4, 14.5.4	Exclusion criterion #7 updated for clarification.
15	Removal of vaccine candidate BNT166c from Substudy C.
15.3.1	Major modification of Substudy C: Removal of sentinel subjects, staggered dosing, visit-specific IRC reviews, and pausing rules from Substudy C design. Initiation of Substudy C is contingent on review of 1 month post-Dose 2 safety, reactogenicity, and immunogenicity data from Substudies A and B for selection of a suitable dose.
15.3.4	Removal of footnote d.
15.4	Correction: Removal of outcome measure "overall for all groups with the same IMP" from safety objective.
15.4	Exploratory objectives: Description of neutralizing antibody responses was changed from an exploratory to a secondary objective.
15.5.4	Modification of exclusion criterion #7.
15.6	Rephrasing of trial treatment information.
16	Introduction of Substudy D.

Abbreviations: AE = adverse event; AESI = adverse event of special interest; CRF = case report form; EDC = electronic data capture; IB = investigator's brochure; IMP = investigational medicinal product; IRC = internal review committee; mpox = monkeypox; SAP = statistical analysis plan; WHO = World Health Organization.

10.8.4 Protocol update from version 4.0 to 5.0

Protocol version 4.0 was updated to version 5.0 to adapt Substudy D: The planned number of trial subjects was updated and an interim analysis was introduced.

See the table for a summary of the reasons for major changes compared to the previous version. Minor editorial changes, such as the correction of typing errors, are not specifically listed.

Section	Reason for change
1.1, 1.2, 9.3, 16.3, 16.3.2	Updated to increase the number of subjects for Substudy D from 25 to 32. This number was chosen to optimize the ability to detect vaccine-induced antigen-specific T-cell responses, based on other recent studies using the same RNA vaccine platform.
9.2	Updated to introduce an interim analysis of Substudy D 1 month post-Dose 2 safety, reactogenicity, and immunogenicity data to inform further clinical development.

10.8.5 Protocol update from version 5.0 to 6.0

Overall update rationale

The update was made to move the assessments of the cell-mediated immune responses and serum cytokine response elicited by BNT166a from the exploratory objectives in Substudy D to additional research analyses.

Other notable changes include the removal of Substudy C from this trial because the comparator vaccine MVA-BN can currently not be sourced. If required, Substudy C can be conducted as a separate trial at a later stage of the BNT166 product development.

Description of changes

Minor editorial changes, such as the correction of typing errors or improvement of language for consistency, are not specifically listed. A comparison of every new sponsor approved protocol version with the next approved version is filed together with the protocol in the TMF.

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

Section	Change and reason for change
Multiple	Updated to reflect major changes in other sections.
Multiple, 15 (previously)	Removal of Substudy C because the comparator vaccine MVA-BN can currently not be sourced. Section numbering in this CTP has therefore shifted: Section 15 (previously dealing with Substudy C) now contains all information specific to Substudy D.
Protocol update – summary of changes	Text and table summarizing the changes of the latest amendment were moved from page 2 to Section 10.8.
1.1, 1.2, 15.3, 15.6	A dose level of 60 µg BNT166a was selected for Substudy D based on all available 1 month post-Dose 2 safety, reactogenicity, and immunogenicity data from Substudies A and B.
1.1, 2.1, 2.2.1	The trial rationale and medical need sections were updated to reflect the current mpox epidemiological situation and available vaccines.
6.6.2	Reference to the Blinding Plan was removed because the internal requirement for blind management plans for sponsor-unblinded trials has been removed.
6.9.1, 6.9.2	Bullets on systemic corticosteroids modified for clarification.
8.3.1	Guidance on physical examinations modified for clarification.
8.3.7	Bullet on assessment of solicited local and systemic reactions deleted due to a content duplication of the first bullet.
8.3.7	Text was clarified regarding e-diary review by the investigator, and regarding site follow-up for missed e-diary entries.
8.7, 8.8, 15.4	Substudy D: cell-mediated immune responses and cytokine response removed from exploratory objectives and added to additional research analyses.
9.5.1, 9.5.2	Minor changes to harmonize wording.
10.1.4	Data protection section updated for clarification on contacting the subject after IMP-related AEs.
10.7.3	Clarification on the definition of solicited events, mainly for data analysis purposes.
13.3.4, 14.3.4, 15.3.4	Substudies A, B, and D: Footnote added to SoAs to clarify the assessments at an Early Termination Visit.
13.4, 14.4, 15.4	Modification of the primary solicited reactogenicity objective: The phrase “collected via subject e-diaries” was removed to allow for the reconstruction of the reactogenicity

Section	Change and reason for change
	symptoms reported as AEs to the clinical events domain (in accordance with the FDA guidance “Submitting Study Datasets for Vaccines to the Office of Vaccines Research and Review”).
15.3.4	Substudy D SoA: Blood volumes for humoral response assessments changed for Visit 10 and Visit 11.

Abbreviations: AE = adverse event; CTP = clinical trial protocol; IMP = investigational medicinal product; MVA-BN = Modified Vaccinia Ankara - Bavarian Nordic; SoA = schedule of activities.

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12 TOXICITY MANAGEMENT/MITIGATION PLANS

12.1 Toxicity management/mitigation plans for local and systemic reactogenicity

Based on experience with other BioNTech RNA-based vaccines and published data from other RNA-based vaccines, it is anticipated that subjects may experience local and systemic (flu-like) reactogenicity events of mostly mild to moderate severity following the administration of RNA vaccines within 1 to 3 d. Symptomology may include injection site pain, fever, chills, rigors, tachycardia, arthralgia, myalgia, lymphadenopathy, headache or nausea.

Treatment of these events is at the discretion of the investigators; however, the following management suggestions are provided:

- After the first occurrence of flu-like symptomatology, subjects can be treated with standard therapeutic dose of acetaminophen (preferable) rather than NSAIDs, according to local guidance after the immunization.
- Corticosteroids should be avoided as either prophylaxis or treatment as it counteracts the effects of immunization.
- Self-limited Grade 3 reactogenicity events may not require discontinuation of future injections in the trial subject, and the local Investigator may discuss with the trial medical monitor.
- Grade 4 reactogenicity events will lead to discontinuation of future injections in the trial subject.

If subjects experience progression of flu-like symptomatology or non-resolution of the symptoms later than 7 d after an IMP dose, the trial medical monitor should be informed.

12.2 Management of risks associated with anemia

This trial limits the total volume drawn 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.

12.3 Management of risks linked to the pandemic COVID-19

Risks linked to the pandemic COVID-19 will be managed by requiring:

- Avoidance of contact with persons tested positive for SARS-CoV-2 or have an increased risk for infection during their participation in the trial.
- Practice of social distancing and follow good practices to reduce their chances of being infected or spreading COVID-19 during their participation in the trial.

- Completion of health status checks which include symptom-directed physical examinations, vital signs assessments, and clinical laboratory tests on visit days.

Use of the subject hotline 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 at the discretion of the investigator.

12.4 Management of symptoms that could represent myocarditis or pericarditis

If any trial subject reports acute chest pain, shortness of breath, palpitations, or other signs or symptoms suggestive of myocarditis or pericarditis within 4 weeks after an IMP dose, refer this subject to a cardiologist for evaluation and management.

For all such cases when myocarditis and/or pericarditis are suspected, the following should be performed:

- An ECG,
- measurement of the troponin I level, and inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate,
- cardiac echocardiogram, and/or
- cardiac magnetic resonance assessments.

For subjects with suspected or confirmed myocarditis and/or pericarditis the treatment will be permanently stopped (see Section 7.1.2).

The confirmed case of myocarditis and/or pericarditis will constitute a treatment pausing rule (see Section 7.1.1).

Subjects should be followed until resolution of symptoms and abnormal test findings.

13 SUBSTUDY A SPECIFIC INFORMATION

13.1 Summary – Substudy A

See Section 1.1 for a synopsis of the overall trial.

See Section 1.2 for a schema depicting the substudy.

See Section 13.3.4 for the SoA for Substudy A.

13.2 Introduction – Substudy A

13.2.1 Substudy background

See Section 2 for an introduction including trial background.

13.2.2 Substudy rationale

Substudy A will investigate the safety, reactogenicity, and immunogenicity of investigational RNA-based multivalent vaccine candidates for active immunization against mpox in healthy subjects with no prior history of known or suspected smallpox vaccination (vaccinia-naïve subjects) at three dose levels.

13.2.3 Benefit/risk assessment

No additional risks are identified for Substudy A beyond those described in Section 2.3.

13.3 Trial design – Substudy A

Substudy A is an open-label, dose-escalation, Phase I substudy to assess the reactogenicity, safety and immunogenicity of up to three dose levels of two multivalent vaccine candidates.

This substudy will enroll ~64 healthy subjects with no prior history of known or suspected smallpox vaccination (vaccinia-naïve subjects). Vaccine candidate BNT166a will be evaluated at 10 (Group A1), 30 (Group A2), and 60 µg (Group A4) dose levels. Vaccine candidate BNT166c will be evaluated at 30 µg dose level (Group A3). Two doses will be given ~31 d apart. The Groups A2 and A3 will be run simultaneously and, therefore, allocation to treatment for these two groups will be randomized. The sponsor may decide not to activate the group with BNT166c (Group A3), in this case there will be no randomization. Accordingly, the sponsor has decided not to activate Group A3. This substudy will inform Substudy D on the preferred dose level.

13.3.1 Staggered dosing in Substudy A

Dosing in Substudy A will be initiated at dose level 10 µg in Group A1 with the dosing of the first four sentinel subjects. These subjects will be dosed at least 1 h apart. Provided that during 48 h after the fourth subject was dosed no group pausing rules (see Section 7.1) are met and there are no safety findings judged by the sponsor or investigator to be of clinical concern, the next 12 subjects will be dosed.

Once all 16 subjects of Group A1 have completed the 7 d follow-up post-Dose 1, all available 7 d reactogenicity and safety data (including symptom-directed physical examination, vital signs, 12-lead ECG, and clinical laboratory data) will be reviewed by the trial IRC. If the trial IRC considers the reactogenicity and safety profile to be acceptable and no pausing rules (see Section 7.1) are met, dosing will be opened for the next dose level (Groups A2 and A3).

Dosing on the next dose level 30 µg will be initiated with four sentinel subjects in in Group A2 and four sentinel subjects in Group A3. These subjects will be dosed at least 1 h apart. Provided that during 48 h after the last subject was dosed no group pausing rules (see Section 7.1) are met and there are no safety findings judged by the sponsor or investigator to be of clinical concern, the next 12 subjects in Group A2 and 12 subjects in Group A3 will be dosed.

Once all 32 subjects of Groups A2 and A3 have completed the 7 d follow-up post-Dose 1, all available 7 d reactogenicity and safety data (including symptom-directed physical examination, vital signs, 12-lead ECG, and clinical laboratory data) will be reviewed by the trial IRC. If the trial IRC considers the reactogenicity and safety profile to be acceptable and no pausing rules (see Section 7.1) are met, dosing will be opened for the next dose level (Group A4).

Dosing on the next dose level 60 µg will be initiated with four sentinel subjects in in Group A4. These subjects will be dosed at least 1 h apart. Provided that during 48 h after the fourth subject was dosed no group pausing rules (see Section 7.1) are met and there are no safety findings judged by the sponsor or investigator to be of clinical concern, the next 12 subjects will be dosed.

Provided no pausing rules (see Section 7.1) are met, subjects who received Dose 1 will proceed to receive Dose 2. If a pausing rule is met, administration of Dose 2 will be paused, pending trial IRC review of all available reactogenicity and safety data, and IRC endorsement of progression to Dose 2.

Trial IRC review may be performed at any timepoint if there are any safety concerns. The IRC may decide to stop IMP administration to other subjects in a particular group.

The conduct of screening activities will be independent of the planned trial IRC reviews.

13.3.2 Planned number of trial subjects – Substudy A

In each group (A1, A2, A3, A4), 16 healthy subjects (see Figure 1).

13.3.3 Duration of all trial periods – Substudy A

This substudy includes a ≤28 d screening period prior to Visit 1, and 12 visits on site spread over ~13 months. For details, see the SoA in Section 13.3.4.

13.3.4 Schedule of activities – Substudy A

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

Visit (V)	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**
Visit description	**Screening**	**Dose 1 (D1)**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose 2 (D2)**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Term. [n]**
Visit day/window: **- relative to D 1**	**1 to 28 d pre-D1**	**1**	**3-4**	**8-10**	**13-17**	**29-33**							
- relative to D 2							**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Collect informed consent	X												
Inclusion / exclusion criteria	X	X (review)											
Medical history incl. prior medication and smallpox vaccination status, and history of mpox, smallpox or vaccinia infection	X	X (update)											
Physical examination	X	X	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]			
Vital signs [b]	X	X (pre- and ~1 h post-dose)	X	X	X	X (pre- and ~1 h post-dose)	X	X	X	X			
Height & body weight	X												
12-lead ECG	X	X	X	X	X	X	X	X	X				
Urine for clinical laboratory [d]	X												

Visit (V)	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Visit description	**Screening**	**Dose 1 (D1)**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose 2 (D2)**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Term. [n]**
Visit day/window:	**1 to 28 d pre-D1**												
- relative to D 1		**1**	**3-4**	**8-10**	**13-17**	**29-33**							
- relative to D 2							**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Blood for viral screen (~14 mL) [e]	X												
Blood for clinical laboratory (9 mL) [f]	X	X [g]		X		X [g]		X		X			
Pregnancy test for VOCBP [h]	X	X				X							
Counsel / remind subjects to perform contraception	X	X	X	X	X	X	X	X	X	X	X		
Blood for humoral response assessments (30 mL) [i]		X [g]		X	X	X [g]		X	X	X	X	X	X
Randomization / allocation to trial treatment		X											
Vaccination and 1 h observation period [c]		X				X							
Injection site assessment		X (pre- and 1 h post-dose)	X	X		X (pre- and 1 h post-dose)	X	X					
Subject hotline availability	Start	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Issue, train, and collect subject e-diaries [j]		Issue, train				Re-train		Collect [j]					Collect (in case of early termination) [j]

Visit (V)	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Visit description	**Screening**	**Dose 1 (D1)**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose 2 (D2)**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Term. [n]**
Visit day/window:	**1 to 28 d pre-D1**												
- relative to D 1		**1**	**3-4**	**8-10**	**13-17**	**29-33**							
- relative to D 2							**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Subjects report local reactions and systemic events (incl. oral body temperature) daily for 7 d after each IMP dose using an e-diary		Start after Dose 1	=>	End after 7 d reporting		Start after Dose 2	=>	End after 7 d reporting					
Investigators review e-diary data daily		Start	=>	End		Start	=>	End					
Record AEs, MAAEs, AESI, and SAEs since last visit		X	X	X	X	X	X	X	X	X	X [k]	X [k]	X [k]
Record pregnancies		X	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Record concomitant medication since last visit		X	X	X	X	X	X	X	X	X	X [l]	X [l]	X [l]
Wellbeing phone call		X [m]				X [m]							
Total volume of blood drawn per visit	**23 mL**	**39 mL**	**0 mL**	**39 mL**	**30 mL**	**39 mL**	**0 mL**	**39 mL**	**30 mL**	**39 mL**	**30 mL**	**30 mL**	**30 mL**

a Brief (symptom-directed) physical examination.

b Vital signs: systolic/diastolic blood pressure, pulse rate, respiratory rate, and oral body temperature. On days with IMP dosing, before and at 1 h (±15 minutes) after dosing.

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

d Dipstick urine analysis. Microscopic urinalysis: if warranted by dipstick results, urine sediment will be microscopically examined.

e Viral screen: screen for human immunodeficiency virus (HIV) 1 and 2, Hepatitis B, and Hepatitis C.

f Clinical laboratory tests: chemistry, hematology. Obtain FSH at Visit 0 only if postmenopausal status needs to be confirmed. Troponin will only be measured in the Screening (V0) sample.

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

h VOCBP: The serum beta human chorionic gonadotropin pregnancy test at Visit 0 will be performed using the sample collected for clinical laboratory tests. Before each dosing, urine pregnancy tests will be performed using a commercial kit and the trial subjects will be counseled about the need for the correct use of a highly effective method of contraception.

i If justified by the collected data, the listed blood draw days and the draw volumes may be adapted if not increasing the total number of draw days or the total volume drawn. Leftover blood after completion of the immunogenicity assessments may be used for additional biomarker analyses.

j Trial site personnel will remind the subjects to record the oral body temperature and the worst grade for each symptom in the e-diary at approximately the same time every evening on the day of IMP dosing and then every day in the evening for a total of seven consecutive days. Also, remind the subject to contact the site if they experience any severe or potentially life-threatening reactogenicity events. At V1 assist the subject with downloading the e-diary application, or issue provisioned device; at V7 or Early Termination Visit, collect e-diary or help the subject to delete application.

k Only record SAEs and AESI since last visit.

l Starting with Visit 10, only prohibited concomitant medications will be recorded.

m Trial subjects contacted by phone will be asked non-leading wellbeing questions at >5 h after IMP administration on the day of dosing and at 24±4 h after each IMP dose.

n At the Early Termination Visit, if possible, all assessments planned for the actual week or day of that visit and which are not included in the Early Termination Visit should also be performed.

Notes:

If, for any reason, subjects are permanently discontinued from the trial before completing all scheduled visits, subjects will complete an Early Termination Visit. If possible, all assessments planned for the actual week or day of that visit as listed in the SoA and which are not included in the Early Termination Visit should also be performed. The only exception will be pregnant women, who will not have further research blood draws, but will otherwise complete planned assessments, if required including pregnancy follow-up as described in Section 10.5.3.

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.

As long as the absolute volume of blood drawn does not change per subject overall, the volume of individual blood draws may be adapted if required, e.g., due to unforeseen shortage of specific tubes. Leftover or back up clinical sample aliquots of a subject, which have the exact same collection times, intended to a particular assessment can be used for another assay than that stated on the label if both assessments are described in the SoA and the subject has consented to all. This is only possible if a primary sample has been used first for its original purpose and it is confirmed that no re-testing is required. Samples can only be used interchangeably if the sample characteristics, collection, shipment, and storage conditions are the same.

Abbreviations: AE = adverse event; CMI = cell-mediated immunity/immune response; d = day(s); D = dose; ECG = 12-lead electrocardiogram; Early Term. = early termination; FU = follow-up (visit); FSH = follicle stimulating hormone; h = hour(s); IMP = investigation medicinal product; mon = month(s); MAAE = medically attended adverse event; SAE = serious adverse event; SoA = schedule of activities; V = visit; VOCBP = volunteers of childbearing potential; wk = week(s); yr = year(s).

13.4 Objectives, outcome measures, and endpoints – Substudy A

OBJECTIVES	OUTCOME MEASURES	ENDPOINTS
Primary objectives		
To describe the reactogenicity after one and two doses of each multivalent vaccine candidate.	For every group: • Proportion (%) of subjects reporting solicited local reactions at the injection site from Dose 1 through Day 7 post-Dose 1 inclusive; and from Dose 2 through Day 7 post-Dose 2 inclusive. • Proportion (%) of subjects reporting solicited systemic events from Dose 1 through Day 7 post-Dose 1 inclusive; and from Dose 2 through Day 7 post-Dose 2 inclusive.	• Solicited local reactions (pain, erythema / redness, induration / swelling). • Solicited systemic events (vomiting, diarrhea, headache, fatigue, myalgia, arthralgia, chills, and fever).
To describe the safety after one and two doses of each multivalent vaccine candidate.	For every group: • Proportion (%) of subjects with at least one unsolicited AE occurring from Dose 1 through Day 28 post-Dose 1 inclusive. • Proportion (%) of subjects with at least one unsolicited AE occurring from Dose 2 through Day 28 post-Dose 2 inclusive. • Proportion (%) of subjects with at least one SAE occurring from Dose 1 through Day 201 post-Dose 1 inclusive. • Proportion (%) of subjects with at least one AESI occurring from Dose 1 through Day 201 post-Dose 1 inclusive.	• Unsolicited AEs, SAEs, and AESIs recorded by the investigator.
Exploratory objectives		
To describe the neutralizing antibody responses against vaccinia and mpox viruses elicited by each multivalent vaccine candidate.	For every group at 2 weeks after Dose 2: • Geometric mean titers. • Geometric means fold rise from baseline (pre-Dose 1). • Proportion (%) of subjects with seroresponse [a].	• Vaccinia-specific neutralizing antibody levels. • MPXV-specific neutralizing antibody levels.
To describe the kinetics and specificities of humoral immune responses induced by vaccination with each multivalent vaccine candidate.	For every group, at all assessed timepoints: • Geometric mean titers.	• Vaccinia-specific and MPXV-specific neutralizing antibody levels. • Multivalent vaccine candidate antigen-specific binding antibody levels.

a. Seroresponse is defined as seropositivity after baseline for subjects who were seronegative at baseline or two-fold or more increase of the antibody titer compared to the baseline titer for subjects with a pre-existing antibody titer (Pittman et al. 2019). For details, see the statistical analysis plan.

Abbreviations: AE = adverse event; AESI = adverse event of special interest; d = day; IMP = investigational medicinal product; MPXV = mpox virus; SAE = serious AE.

13.5 Trial population – Substudy A

13.5.1 Selection of trial population

The trial population will be healthy subjects with no prior history of known or suspected smallpox vaccination (vaccinia-naïve subjects).

13.5.2 Rationale for trial population

The selection of healthy adult male and female subjects for FIH clinical trials is standard practice to minimize the potential risks which might exist in the subjects with comorbidities and also in subjects taking concomitant medications or who need additional interventions for the underlying disease.

The subjects who have not received any Orthopoxvirus vaccine in the past will take part in Substudy A to investigate the safety and immune response in this group representing majority of the world population who did not have Orthopoxvirus vaccination.

13.5.3 Inclusion criteria

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

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

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

3 Are 18 through 45 years of age (inclusive) at the time of informed consent.

4 Have a body mass index over 18.5 kg/m^2 and under 30 kg/m^2 and weigh at least 50 kg at Visit 0.

5 Are healthy, in the clinical judgment of the investigator based on volunteer-reported medical history data, physical examination, 12-lead ECG, vital signs, and clinical laboratory test results.

6 Have no prior history of known or suspected smallpox vaccination and no detectable smallpox vaccination characteristic scar (vaccinia-naïve subjects).

7 Agree not to enroll in another trial with an IMP starting from Visit 0 and until the end of this trial (Visit 12).

Virology

8 Negative HIV-1 and HIV-2 antigen/antibody blood test result at Visit 0.

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

Reproductive status and contraception

10 VOCBP that have a negative serum 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 (for definitions of postmenopausal and permanently sterilized, see Section 10.5.1).

11 VOCBP who have used a highly effective form of contraception for at least 28 d prior to Dose 1 and agree to practice this method of contraception continuously for 90 d after Dose 2. For guidance on contraception see Section 10.5.2).

12 VOCBP who agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction during trial, starting at Visit 0 and continuously for 90 d after Dose 2.

13 Men who are sexually active with partners of childbearing potential and who have not had a vasectomy that agree to practice a highly effective form of contraception with their sexual partners born female during the trial, starting at Visit 0 and continuously for 90 d after Dose 2 (for guidance on contraception, see Section 10.5.2).

14 Men who are willing to refrain from sperm donation, starting at Visit 0 and continuously for 90 d after Dose 2.

13.5.4 Exclusion criteria

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

1 History of mpox, smallpox or vaccinia infection based on volunteer-reported medical history.

2 Pregnant, breastfeeding, planning pregnancy or planning to father children starting from Visit 0 and continuously until 90 d after receiving Dose 2.

3 History of known or suspected severe adverse reaction including allergic reaction (e.g., anaphylaxis) to vaccines or to vaccine components such as lipids.

4 Current or history of the following medical conditions at Visit 0 or Visit 1:

a) Uncontrolled, moderate or severe asthma; asthma severity as defined in the US National Asthma Education and Prevention Program Expert Panel report, e.g., exclude volunteers who:

- Use a short-acting rescue inhaler (typically a beta 2 agonist) daily, or
- Use high dose inhaled corticosteroids (per American Academy of Allergy Asthma & Immunology), or
- In the past year where any of the following apply:

- Greater than one exacerbation of symptoms requiring oral/parenteral corticosteroids treatment.
- Needed hospitalization or intubation for asthma.

b) Chronic obstructive pulmonary disease.

c) Diabetes mellitus type 1 or type 2, including cases controlled with diet alone (Not excluded: history of isolated gestational diabetes).

d) Hypertension: If a person has hypertension, 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.

e) Systolic blood pressure ≥150 mm Hg or diastolic blood pressure ≥100 mm Hg.

f) Malignancy, excluding localized basal or squamous cell cancer.

g) Cardiovascular diseases, (e.g., myocarditis, pericarditis, coronary heart disease, myocardial infarction, congestive heart failure, cardiomyopathy or clinically significant arrhythmias, stroke or transient ischemic attack).

h) Bleeding disorders (e.g., factor deficiency, coagulopathy, or platelet disorder).

i) Seizure disorder: History of seizure(s) within past 3 years; use of medications in order to prevent or treat seizure(s) at any time within the past 3 years.

j) Estimated glomerular filtration rate <60 mL/min/1.73m^2.

k) Chronic liver disease.

5 Schizophrenia, major depressive disorder, suicidal ideation. Such psychiatric illnesses, as bipolar disorder, 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.

6 Current or history of the following diseases associated with immune dysregulation:

- Known or suspected immunodeficiency.
- History of solid organ or bone marrow transplantation.
- Asplenia: any condition resulting in the absence of a functional spleen.
- Currently existing or history of any autoimmune disease.

7 At Visit 0, any screening hematology and/or blood chemistry laboratory value that meets the definition of a Grade ≥1 abnormality (according to the FDA toxicity grading scale; see separate exclusion criteria for bilirubin and troponin I). Individuals with any stable Grade 1 abnormalities may be considered

eligible at the discretion of the investigator. A stable Grade 1 laboratory abnormality is defined as the value which is ≤ Grade 1 upon repeated testing on a second sample from this individual during the screening period (prior to Visit 1). Individuals with abnormal but not clinically significant parameters (for definition of clinical significance see Section 10.4.1.3.2) not included in the FDA toxicity guidance may be considered eligible at discretion of investigator.

8 Abnormal total bilirubin at Visit 0. Note: inclusion of volunteers with bilirubin ≤1.25 ULN if due to Gilbert's syndrome is allowed.

9 Any abnormal troponin I value at Visit 0.

10 A 12-lead ECG at Visit 0 which is consistent with probable or possible myocarditis/pericarditis or which demonstrates clinically relevant abnormalities that may affect subject safety or are otherwise clinically significant findings (e.g., complete left bundle branch block, AV block, average QTcF interval >450 msec, signs of myocardial infarction, ST elevation consistent with myocardial ischemia, or serious brady- or tachyarrhythmias).

11 Febrile illness (body temperature ≥38.0°C) or other acute illness within 48 h prior to Dose 1 and/or current (if presented at Visit 1, temporary deferral is allowed).

12 Participation or planned participation in strenuous or endurance exercise (see Section 5.5 for a definition) within 7 d before or after each IMP administration.

Prior/concomitant therapy

13 Vaccination with any Orthopoxvirus-based vaccine including vaccines for prevention of smallpox, disease caused by vaccinia virus or mpox, or vector Orthopoxvirus-based vaccines.

14 Any vaccination within 28 d before Dose 1. Seasonal inactivated influenza vaccine is allowed, however, it should be administered at least 14 d before IMP administration.

15 Any non-trial IMP within 28 d or five half-lives (whichever is longer) before Dose 1.

16 Blood/plasma products and/or immunoglobulins within 120 d before Dose 1.

17 Allergy treatment with antigen injections within 14 d before Dose 1.

18 Immunosuppressive therapy, including corticosteroids, or radiotherapy within 6 months or five half-lives (whichever is longer) before Dose 1. If systemic corticosteroids have been administered short term (≤14 d, at a dose of ≤20 mg/d of prednisone or equivalent) for treatment of an acute illness, individuals should be enrolled in the trial only after corticosteroid therapy has been discontinued for at least 28 d before Dose 1. Intraarticular, intrabursal, or topical (skin or eyes) corticosteroids are permitted.

Additional exclusions

19 Have a history of alcohol abuse within 1 year before Visit 0 or have a history of substance abuse within the past 5 years before Visit 0.

20 Are vulnerable individuals as per 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.

13.6 Trial treatment – Substudy A

Use	Investigational
IMP name	Multivalent vaccine candidates: BNT166a, BNT166c
Type	RNA-based mpox vaccines. Each multivalent vaccine candidate includes up to four monovalent RNA-LNP components (monovalent DP). Each monovalent DP contains an LNP-encapsulated RNA encoding mpox virus protein (antigen). Each RNA is purified single-stranded, 5'-capped N1-methylpseudouridine modified ("nucleoside-modified") RNA produced by *in vitro* transcription from a DNA template. Two candidates are listed below together with the mpox antigens targeted by each included monovalent DP: • BNT166a: antigens B6, A35, M1, and H3. • BNT166c: antigens B6, A35, and M1.
Dose levels	BNT166a: 10 µg, 30 µg, and 60 µg BNT166c: 30 µg
Dosing route and regimen	Two intramuscular doses of the multivalent vaccine candidate, one at Visit 1 (Day 1) and one at Visit 5 (Day 31). Injections should be in the deltoid muscle, using the same non-dominant arm for all IMP doses.
IMP preparation	Trial sites will be provided with the monovalent DP. Administration of each multivalent vaccine candidate will be performed by simultaneous (one injection) intramuscular administration of the required monovalent DPs after mixing at the trial site. See the Pharmacy Manual for guidance for the preparation of the IMP.
Source	BioNTech

Abbreviations: DNA = deoxyribonucleic acid; DP = drug product; LNP = lipid nanoparticle; IMP = investigational medicinal product; RNA = ribonucleic acid.

All provided IMP will be labeled as required per country requirements (see the Pharmacy Manual for details).

13.7 Statistical considerations – Substudy A

See Section 9.

14 SUBSTUDY B SPECIFIC INFORMATION

14.1 Summary – Substudy B

See Section 1.1 for a synopsis of the overall trial.

See Section 1.2 for a schema depicting the substudy.

See Section 14.3.4 for the SoA for Substudy B.

14.2 Introduction – Substudy B

14.2.1 Substudy background

See Section 2 for an introduction including trial background.

14.2.2 Substudy rationale

Substudy B will investigate the safety, reactogenicity, and immunogenicity of investigational RNA-based multivalent vaccine candidates for active immunization against mpox in healthy subjects with prior history of smallpox vaccination (vaccinia-experienced subjects).

14.2.3 Benefit/risk assessment

No additional risks are identified for Substudy B beyond those described in Section 2.3.

14.3 Trial design – Substudy B

Substudy B is a randomized, observer-blinded and sponsor-unblinded Phase I substudy to assess the reactogenicity, safety and immunogenicity of two multivalent vaccine candidates. This substudy will enroll ~32 healthy subjects with prior history of smallpox vaccination (vaccinia-experienced). The smallpox vaccination status will be determined based on the medical record of vaccination before 1980 or the presence of the smallpox vaccination characteristic scar. Both multivalent vaccine candidates will be evaluated at 30 µg dose levels, two doses will be given ~31 d apart. Subjects will be randomized 1:1. The sponsor may decide not to activate one of the groups, in that case this substudy will be a one group open-label substudy. Accordingly, the sponsor has decided not to activate Group B2, which was planned to be dosed with vaccine candidate BNT166c.

14.3.1 Staggered dosing in Substudy B

Dosing in Substudy B will be initiated once the trial IRC deems the 7 d follow-up post-Dose 1 reactogenicity and safety data of the 10 µg Group of Substudy A subjects are acceptable. The first four sentinel subjects (two subjects in each group) will be dosed at least 1 h apart.

Provided that during 48 h after the last subject was dosed no group pausing rules (see Section 7.1) are met and there are no safety findings judged by sponsor or investigator to be of clinical concern, the next four subjects will be dosed.

Once the first eight subjects have completed the 7 d follow-up post-Dose 1, all available 7 d reactogenicity and safety data (including symptom-directed physical examination, vital signs, 12-lead ECG, and clinical laboratory data) will be reviewed by the trial IRC. If the trial IRC considers the reactogenicity and safety profile to be acceptable and no pausing rules (see Section 7.1) are met, dosing of the next 24 subjects will start.

Provided no pausing rules (see Section 7.1) are met, subjects who received Dose 1 will proceed to receive Dose 2. If a pausing rule is met, administration of Dose 2 will be paused, pending trial IRC review of all available reactogenicity and safety data, and trial IRC endorsement of progression to Dose 2.

Trial IRC review may be performed at any timepoint if there are any safety concerns. The trial IRC may decide to unblind the treatment for affected subject(s), and/or to stop IMP administration to other subjects in a particular group.

The conduct of screening activities will be independent of the planned trial IRC reviews.

14.3.2 Planned number of trial subjects – Substudy B

In each group (B1 and B2), 16 healthy subjects (see Figure 1).

14.3.3 Duration of all trial periods – Substudy B

This substudy includes a ≤28 d screening period prior to Visit 1, and 12 visits on site spread over ~13 months. For details, see the SoA in Section 14.3.4.

14.3.4 Schedule of activities – Substudy B

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

Visit (V)	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**
Visit description	**Screening**	**Dose (D) 1**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose (D) 2**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Termination [n]**
Visit day/window:	**1 to 28 d pre-D1**												
- relative to Dose 1		**1**	**3-4**	**8-10**	**13-17**	**29-33**							
- relative to Dose 2							**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Collect informed consent	X												
Inclusion / exclusion criteria	X	X (review)											
Medical history incl. prior medication and smallpox vaccination status, and history of mpox, smallpox or vaccinia infection	X	X (update)											
Physical examination	X	X	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]			
Vital signs [b]	X	X (pre- and ~1 h post-dose)	X	X	X	X (pre- and ~1 h post-dose)	X	X	X	X			
Height & body weight	X												
12-lead ECG	X	X	X	X	X	X	X	X	X				
Urine for clinical laboratory [d]	X												

Visit (V)	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Visit description	**Screening**	**Dose (D) 1**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose (D) 2**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Termination [n]**
Visit day/window:	**1 to 28 d pre-D1**												
- relative to Dose 1		**1**	**3-4**	**8-10**	**13-17**	**29-33**							
- relative to Dose 2							**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Blood for viral screen (~14 mL) [e]	X												
Blood for clinical laboratory (9 mL) [f]	X	X [g]		X		X [g]		X		X			
Pregnancy test for VOCBP [h]	X	X				X							
Counsel / remind subjects to perform contraception	X	X	X	X	X	X	X	X	X	X	X		
Blood for humoral response assessments (30 mL) [i]		X [g]		X	X	X [g]		X	X	X	X	X	X
Randomization / allocation to trial treatment		X											
Vaccination and 1 h observation period [c]		X				X							
Injection site assessment		X (pre- and 1 h post-dose)	X	X		X (pre- and 1 h post-dose)	X	X					
Subject hotline availability	Start	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End

Visit (V)	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Visit description	**Screening**	**Dose (D) 1**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose (D) 2**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Termination [n]**
Visit day/window:	**1 to 28 d pre-D1**												
- relative to Dose 1		**1**	**3-4**	**8-10**	**13-17**	**29-33**							
- relative to Dose 2							**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Issue, train, and collect subject e-diaries [j]		Issue, train				Re-train		Collect [j]					Collect (in case of early termination) [j]
Subjects report local reactions and systemic events (incl. oral body temperature) daily for 7 d after each IMP dose using an e-diary		Start after Dose 1	=>	End after 7 d reporting		Start after Dose 2	=>	End after 7 d reporting					
Investigators review e-diary data daily		Start	=>	End		Start	=>	End					
Record AEs, MAAEs, AESI, and SAEs since last visit		X	X	X	X	X	X	X	X	X	X [k]	X [k]	X [k]
Record pregnancies		X	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Record concomitant medication since last visit		X	X	X	X	X	X	X	X	X	X [l]	X [l]	X [l]
Wellbeing phone call		X [m]				X [m]							
Total volume of blood drawn per visit	**23 mL**	**39 mL**	**0 mL**	**39 mL**	**30 mL**	**39 mL**	**0 mL**	**39 mL**	**30 mL**	**39 mL**	**30 mL**	**30 mL**	**30 mL**

a Brief (symptom-directed) physical examination.

b Vital signs: systolic/diastolic blood pressure, pulse rate, respiratory rate, and oral body temperature. On days with IMP dosing, before and at 1 h (±15 minutes) after dosing.

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

d Dipstick urine analysis. Microscopic urinalysis: if warranted by dipstick results, urine sediment will be microscopically examined.

e Viral screen: screen for human immunodeficiency virus (HIV) 1 and 2, Hepatitis B, and Hepatitis C.

f Clinical laboratory tests: chemistry, hematology. Obtain FSH at Visit 0 only if postmenopausal status needs to be confirmed. Troponin will only be measured in the Screening (V0) sample.

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

h VOCBP: The serum beta human chorionic gonadotropin pregnancy test at Visit 0 will be performed using the sample collected for clinical laboratory tests. Before each dosing, urine pregnancy tests will be performed using a commercial kit and the trial subjects will be counseled about the need for the correct use of a highly effective method of contraception.

i If justified by the collected data, the listed blood draw days and the draw volumes may be adapted if not increasing the total number of draw days or the total volume drawn. Leftover blood after completion of the immunogenicity assessments may be used for additional biomarker analyses.

j Trial site personnel will remind the subjects to record the oral body temperature and the worst grade for each symptom in the e-diary at approximately the same time every evening on the day of IMP dosing and then every day in the evening for a total of seven consecutive days. Also, remind the subject to contact the site if they experience any severe or potentially life-threatening reactogenicity events. At V1 assist the subject with downloading the e-diary application, or issue provisioned device; at V7 or Early Termination Visit, collect e-diary or help the subject to delete application.

k Only record SAEs and AESI since last visit.

l Starting with Visit 10, only prohibited concomitant medications will be recorded.

m Trial subjects contacted by phone will be asked non-leading wellbeing questions >5 h after IMP administration on the day of dosing and at 24±4 h after each IMP dose.

n At the Early Termination Visit, if possible, all assessments planned for the actual week or day of that visit and which are not included in the Early Termination Visit should also be performed.

Notes:

If, for any reason, subjects are permanently discontinued from the trial before completing all scheduled visits, subjects will complete an Early Termination Visit. If possible, all assessments planned for the actual week or day of that visit as listed in the SoA and which are not included in the Early Termination Visit should also be performed. The only exception will be pregnant women, who will not have further research blood draws, but will otherwise complete planned assessments, if required including pregnancy follow-up as described in Section 10.5.3.

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.

As long as the absolute volume of blood drawn does not change per subject overall, the volume of individual blood draws may be adapted if required, e.g., due to unforeseen shortage of specific tubes. Leftover or back up clinical sample aliquots of a subject, which have the exact same collection times, intended to a particular assessment can be used for another assay than that stated on the label if both assessments are described in the SoA and the subject has consented to all. This is only possible if a primary sample has been used first for its original purpose and it is confirmed that no re-testing is required. Samples can only be used interchangeably if the sample characteristics, collection, shipment, and storage conditions are the same.

Abbreviations: AE = adverse event; CMI = cell-mediated immunity/immune response; d = day(s); D = dose; ECG = 12-lead electrocardiogram; Early Term. = early termination; FU = follow-up (visit); FSH = follicle stimulating hormone; h = hour(s); IMP = investigation medicinal product; MAAE = medically attended adverse event; mon = month(s); SAE = serious adverse event; SoA = schedule of activities; V = visit; VOCBP = volunteers of childbearing potential; wk = week(s); yr = year(s).

14.4 Objectives, outcome measures, and endpoints – Substudy B

OBJECTIVES	OUTCOME MEASURES	ENDPOINTS
Primary objectives		
To describe the reactogenicity after one and two doses of each multivalent vaccine candidate.	For every group: • Proportion (%) of subjects reporting solicited local reactions at the injection site from Dose 1 through Day 7 post-Dose 1 inclusive; and from Dose 2 through Day 7 post-Dose 2 inclusive. • Proportion (%) of subjects reporting solicited systemic events from Dose 1 through Day 7 post-Dose 1 inclusive; and from Dose 2 through Day 7 post-Dose 2 inclusive.	• Solicited local reactions (pain, erythema / redness, induration / swelling). • Solicited systemic events (vomiting, diarrhea, headache, fatigue, myalgia, arthralgia, chills, and fever).
To describe the safety after one and two doses of each multivalent vaccine candidate.	For every group: • Proportion (%) of subjects with at least one unsolicited AE occurring from Dose 1 through Day 28 post-Dose 1 inclusive. • Proportion (%) of subjects with at least one unsolicited AE occurring from Dose 2 through Day 28 post-Dose 2 inclusive. • Proportion (%) of subjects with at least one SAE occurring from Dose 1 through Day 201 post-Dose 1 inclusive. • Proportion (%) of subjects with at least one AESI occurring from Dose 1 through Day 201 post-Dose 1 inclusive.	• Unsolicited AEs, SAEs, and AESIs recorded by the investigator.
Exploratory objectives		
To describe the neutralizing antibody responses against vaccinia and mpox viruses elicited by each multivalent vaccine candidate.	For every group at 2 weeks after Dose 2: • Geometric mean titers. • Geometric means fold rise from baseline (pre-Dose 1). • Proportion (%) of subjects with seroresponse [a].	• Vaccinia-specific neutralizing antibody levels. • MPXV-specific neutralizing antibody levels.
To describe the kinetics and specificities of humoral immune responses induced by vaccination with each multivalent vaccine candidate.	For every group, at all assessed timepoints: • Geometric mean titers.	• Vaccinia-specific and MPXV-specific neutralizing antibody levels. • Multivalent vaccine candidate antigen-specific binding antibody levels.

a Seroresponse is defined as seropositivity after baseline for subjects who were seronegative at baseline or two-fold or more increase of the antibody titer compared to the baseline titer for subjects with a pre-existing antibody titer (Pittman et al. 2019). For details, see the statistical analysis plan.

Abbreviations: AE = adverse event; AESI = adverse event of special interest; d = day(s); IMP = investigational medicinal product; MPXV = mpox virus; SAE = serious AE.

14.5 Trial population – Substudy B

14.5.1 Selection of trial population

The inclusion of healthy subjects with prior history of smallpox vaccination* (vaccinia-experienced subjects) in Substudy B.

14.5.2 Rationale for trial population

The selection of healthy adult male and female subjects for FIH clinical trials is the standard practice to minimize the potential risks which might exist in the subjects with comorbidities and also in subjects taking concomitant medications or who need additional interventions for the underlying disease.

Subjects who have been vaccinated against smallpox before 1980 will take part in Substudy B to allow investigation of the safety and immune response in people who potentially have immunity to vaccinia virus and therefore might have some level of immunity to mpox.

14.5.3 Inclusion criteria

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

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

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

3 Are 50 through 65 years of age (inclusive) at the time of informed consent.

4 Have a body mass index over 18.5 kg/m^2 and under 30 kg/m^2 and weigh at least 50 kg at Visit 0.

5 Are healthy, in the clinical judgment of the investigator based on volunteer-reported medical history data, physical examination, 12-lead ECG, vital signs, and clinical laboratory test results.

6 Have a history of prior smallpox vaccination (i.e., are vaccinia-experienced), determined based on medical records and/or presence of smallpox vaccination characteristic scar. The most recent smallpox vaccination should have been received before 1980.

7 Agree not to enroll in another trial with an IMP starting from Visit 0 and until the end of this trial (Visit 12).

Virology

8 Negative HIV-1 and HIV-2 antigen/antibody blood test result at Visit 0.

9 Negative Hepatitis B surface antigen and negative core antibodies test results and negative anti-HCV, or negative HCV PCR test result if the anti-HCV is positive at Visit 0.

Reproductive status and contraception

10 VOCBP that have a negative serum 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 (for definitions of postmenopausal and permanently sterilized, see Section 10.5.1).

11 VOCBP who have used a highly effective form of contraception for at least 28 d prior to Dose 1 and agree to practice this method of contraception continuously for 90 d after Dose 2. For guidance on contraception see Section 10.5.2).

12 VOCBP who agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction during trial, starting at Visit 0 and continuously for 90 d after Dose 2.

13 Men who are sexually active with partners of childbearing potential and who have not had a vasectomy that agree to practice a highly effective form of contraception with their sexual partners born female during the trial, starting at Visit 0 and continuously for 90 d after Dose 2 (for guidance on contraception, see Section 10.5.2).

14 Men who are willing to refrain from sperm donation, starting at Visit 0 and continuously for 90 d after Dose 2.

14.5.4 Exclusion criteria

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

1 History of mpox, smallpox or vaccinia infection based on volunteer-reported medical history.

2 Pregnant, breastfeeding, planning pregnancy or planning to father children starting from Visit 0 and continuously until 90 d after receiving Dose 2.

3 History of known or suspected severe adverse reaction including allergic reaction (e.g., anaphylaxis) to vaccines or to vaccine components such as lipids.

4 Current or history of the following medical conditions at Visit 0 or Visit 1:

a) Uncontrolled, moderate or severe asthma; asthma severity as defined in the US National Asthma Education and Prevention Program Expert Panel report, e.g., exclude volunteers who:

- Use a short-acting rescue inhaler (typically a beta 2 agonist) daily, or
- Use high dose inhaled corticosteroids (per American Academy of Allergy Asthma & Immunology), or
- In the past year where any of the following apply:
  - Greater than one exacerbation of symptoms requiring oral/parenteral corticosteroids treatment.
  - Needed hospitalization or intubation for asthma.

b) Chronic obstructive pulmonary disease.

c) Diabetes mellitus type 1 or type 2, including cases controlled with diet alone (Not excluded: history of isolated gestational diabetes).

d) Hypertension: If a person has hypertension, 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.

e) Systolic blood pressure ≥150 mm Hg or diastolic blood pressure ≥100 mm Hg.

f) Malignancy, excluding localized basal or squamous cell cancer.

g) Cardiovascular diseases, (e.g., myocarditis, pericarditis, coronary heart disease, myocardial infarction, congestive heart failure, cardiomyopathy or clinically significant arrhythmias, stroke or transient ischemic attack).

h) Bleeding disorders (e.g., factor deficiency, coagulopathy, or platelet disorder).

i) Seizure disorder: History of seizure(s) within past 3 years; use of medications in order to prevent or treat seizure(s) at any time within the past 3 years.

j) Estimated glomerular filtration rate <60 mL/min/1.73m^2.

k) Chronic liver disease.

5 Schizophrenia, major depressive disorder, suicidal ideation. Such psychiatric illnesses, as bipolar disorder, 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.

6 Current or history of the following diseases associated with immune dysregulation:

- Known or suspected immunodeficiency.
- History of solid organ or bone marrow transplantation.
- Asplenia: any condition resulting in the absence of a functional spleen.
- Currently existing or history of any autoimmune disease.

7 At Visit 0, any screening hematology and/or blood chemistry laboratory value that meets the definition of a Grade ≥1 abnormality at screening (according to the FDA toxicity grading scale; see separate exclusion criteria for bilirubin and troponin I). Individuals with any stable Grade 1 abnormalities may be considered eligible at the discretion of the investigator. A stable Grade 1 laboratory abnormality is defined as the value which is ≤ Grade 1 upon repeated testing on a second sample from this individual during the screening period (prior to Visit 1). Individuals with abnormal but not clinically significant parameters (for definition of clinical significance see Section 10.4.1.3.2) not included in the FDA toxicity guidance may be considered eligible at discretion of investigator.

8 Abnormal total bilirubin at Visit 0. Note: inclusion of volunteers with bilirubin ≤1.25 ULN if due to Gilbert's syndrome is allowed.

9 Any abnormal troponin I value at Visit 0.

10 A 12-lead ECG at Visit 0 which is consistent with probable or possible myocarditis/pericarditis or which demonstrates clinically relevant abnormalities that may affect subject safety or are otherwise clinically significant findings (e.g. complete left bundle branch block, AV block, average QTcF interval >450 msec, signs of myocardial infarction, ST elevation consistent with myocardial ischemia, or serious brady- or tachyarrhythmias).

11 Febrile illness (body temperature ≥38.0°C) or other acute illness within 48 h prior to Dose 1 and/or current (if presented at Visit 1, temporary deferral is allowed).

12 Participation or planned participation in strenuous or endurance exercise (see Section 5.5 for a definition) within 7 d before or after each IMP administration.

Prior/concomitant therapy

13 Vaccination for prevention of mpox or disease caused by vaccinia virus, or with vector Orthopoxvirus-based vaccine. Vaccination for prevention of smallpox done in or after 1980.

14 Any vaccination within 28 d before Dose 1. Seasonal inactivated influenza vaccine is allowed, however, it should be administered at least 14 d before IMP administration.

15 Any non-trial IMP within 28 d or five half-lives (whichever is longer) before Dose 1.

16 Blood/plasma products and/or immunoglobulins within 120 d before Dose 1.

17 Allergy treatment with antigen injections within 14 d before Dose 1.

18 Immunosuppressive therapy, including corticosteroids, or radiotherapy within 6 months or five half-lives (whichever is longer) before Dose 1. If systemic corticosteroids have been administered short term (≤14 d, at a dose of ≤20 mg/d of prednisone or equivalent) for treatment of an acute illness, individuals should be enrolled in the trial only after corticosteroid therapy has been discontinued for at least 28 d before Dose 1. Intraarticular, intrabursal, or topical (skin or eyes) corticosteroids are permitted.

Additional exclusions

19 Have a history of alcohol abuse within 1 year before Visit 0 or have a history of substance abuse within the past 5 years before Visit 0.

20 Are vulnerable individuals as per 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.

14.6 Trial treatment – Substudy B

Use	Investigational
IMP name	Multivalent vaccine candidates: BNT166a, BNT166c
Type	RNA-based mpox vaccines. Each multivalent vaccine candidate includes up to four monovalent RNA-LNP components (monovalent DP). Each monovalent DP contains an LNP-encapsulated RNA encoding mpox virus protein (antigen). Each RNA is purified single-stranded, 5'-capped N1-methylpseudouridine modified ("nucleoside-modified") RNA produced by *in vitro* transcription from a DNA template. The two candidates are listed below together with the mpox antigens targeted by each included monovalent DP: • BNT166a: antigens B6, A35, M1, and H3. • BNT166c: antigens B6, A35, and M1.
Dose levels	30 µg
Dosing route and regimen	Two intramuscular doses of the multivalent vaccine candidate, one at Visit 1 (Day 1) and one at Visit 5 (Day 31). Injections should be in the deltoid muscle, using the same non-dominant arm for all IMP doses.
IMP preparation	Trial sites will be provided with the monovalent DP. Administration of each multivalent vaccine candidate will be performed by simultaneous (one injection) intramuscular administration of the required monovalent DPs after mixing at the trial site. See the Pharmacy Manual for guidance for the preparation of the IMP.
Source	BioNTech

Abbreviations: DNA = deoxyribonucleic acid; DP = drug product; IMP = investigational medicinal product; LNP = lipid nanoparticle; RNA = ribonucleic acid.

All provided IMP will be labeled as required per country requirements (see the Pharmacy Manual for details).

14.7 Statistical considerations – Substudy B

See Section 9.

15 SUBSTUDY D SPECIFIC INFORMATION

15.1 Summary – Substudy D

See Section 1.1 for a synopsis of the overall trial.

See Section 1.2 for a schema depicting the substudy.

See Section 15.3.4 for the SoA for Substudy D.

15.2 Introduction – Substudy D

15.2.1 Substudy background

See Section 2 for an introduction including trial background.

15.2.2 Substudy rationale

Substudy D will investigate the safety, reactogenicity, and immunogenicity of the investigational RNA-based multivalent vaccine candidate BNT166a for active immunization against mpox in healthy subjects with no prior history of known or suspected smallpox vaccination (vaccinia-naïve subjects). This substudy will further investigate and characterize the immunogenicity of BNT166a by assessing not only binding and neutralizing antibodies, but also functional antibody responses, cell-mediated immunity, and innate immunity.

15.2.3 Benefit/risk assessment

No additional risks are identified for Substudy D beyond those described in Section 2.3.

15.3 Trial design – Substudy D

Substudy D is a one group, open-label, Phase IIa substudy to assess the safety, reactogenicity, and immunogenicity of one dose level of the multivalent vaccine candidate BNT166a.

This substudy will enroll ~32 healthy subjects with no prior history of known or suspected smallpox vaccination (vaccinia-naïve subjects). Vaccine candidate BNT166a will be evaluated at a dose level of 60 µg. Two doses will be given ~31 d apart.

15.3.1 Dosing in Substudy D

Substudy D will be initiated after the interim analysis of Substudies A and B 1 month post-Dose 2 safety, reactogenicity, and immunogenicity data (from all subjects who completed their Visit 9). This interim analysis will inform on the dose for Substudy D. If the safety, reactogenicity, and immunogenicity profile was assessed as acceptable and no pausing rules (see Section 7.1) were met in Substudies A and B, Substudy D may be initiated.

Trial IRC review will continue to be performed on a regular basis and may be performed at any timepoint if there are any safety concerns. The trial IRC may decide to stop IMP administration to other subjects.

15.3.2 Planned number of trial subjects – Substudy D

In Substudy D, 32 healthy subjects will be enrolled (see Figure 1).

15.3.3 Duration of all trial periods – Substudy D

This substudy includes a ≤28 d screening period prior to Visit 1, and 14 visits on site spread over ~13 months. For details, see the SoA in Section 15.3.4.

15.3.4 Schedule of activities – Substudy D

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

Visit (V)	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**
Visit description	**Scre en-ing**	**Dose 1 (D1)**	**1 d after D1**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose 2 (D2)**	**1 d after D2**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Term. [n]**
Visit day/window: **- relative to D1**	**1 to 28 d pre-D1**	**1**	**2**	**3-4**	**8-10**	**13-17**	**29-33**								
- relative to D2							**1**	**2**	**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Collect informed consent	X														
Inclusion / exclusion criteria	X	X (review)													
Medical history incl. prior medication and smallpox vaccination status, and history of mpox, smallpox or vaccinia infection	X	X (update)													
Physical examination	X	X	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]	X [a]
Vital signs [b]	X	X (pre- and ~1 h post-dose)	X	X	X	X	X (pre- and ~1 h post-dose)	X	X	X	X	X			

Visit (V)	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
Visit description	**Screen-ing**	**Dose 1 (D1)**	**1 d after D1**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose 2 (D2)**	**1 d after D2**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Term. [n]**
Visit day/window: **- relative to D1**	**1 to 28 d pre-D1**	**1**	**2**	**3-4**	**8-10**	**13-17**	**29-33**								
- relative to D2							**1**	**2**	**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Height & body weight	X														
12-lead ECG	X	X		X	X	X	X		X	X	X				
Urine for clinical laboratory	X														
Blood for viral screen (~14 mL) [e]	X														
Blood for clinical laboratory (9 mL) [f]	X	X [g]			X		X [g]			X		X			
Pregnancy test for VOCBP [h]	X	X					X								
Blood for HLA typing (5 mL)		X [g]													
Blood for humoral response assessments (15 mL) [i]		X [g] (30 mL)					X [g]			X	X	X (30 mL)	X (30 mL)	X	X
Blood for CMI research (80 mL)		X [g]					X [g]			X		X	X	X	X
Blood for transcriptome analysis (5 mL)		X [g]	X	X			X [g]	X	X	X					

Visit (V)	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
Visit description	**Scre en-ing**	**Dose 1 (D1)**	**1 d after D1**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose 2 (D2)**	**1 d after D2**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Term. [n]**
Visit day/window: **- relative to D1**	**1 to 28 d pre-D1**	**1**	**2**	**3-4**	**8-10**	**13-17**	**29-33**								
- relative to D2							**1**	**2**	**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Blood for cytokine assessment (5 mL)		X [g]	X	X			X [g]	X	X						
Allocation to trial treatment		X													
Vaccination and 1 h observation period [c]		X					X								
Injection site assessment		X (pre- and >1 h post-dose)	X	X	X		X (pre- and >1 h post-dose)	X	X	X					
Issue, train, and collect subject e-diaries [j]		Issue, train			Collect		Re-train			Collect [j]					Collect (in case of early termina-tion) [j]

Visit (V)	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**
Visit description	**Scre en-ing**	**Dose 1 (D1)**	**1 d after D1**	**3 d after D1**	**1 wk after D1**	**2 wk after D1**	**Dose 2 (D2)**	**1 d after D2**	**3 d after D2**	**1 wk after D2**	**2 wk after D2**	**1 mon after D2**	**3 mon after D2**	**6 mon after D2**	**1 yr after D2 or Early Term. [n]**
Visit day/window: **- relative to D1**	**1 to 28 d pre-D1**	**1**	**2**	**3-4**	**8-10**	**13-17**	**29-33**								
- relative to D2							**1**	**2**	**3-4**	**8-10**	**13-17**	**29-33**	**86-96**	**171-191**	**356-376**
Subjects report local reactions and systemic events (incl. oral body temperature) daily for 7 d after each IMP dose using an e-diary		Start after Dose 1	=>	=>	End after 7 d repor-ting		Start after Dose 2	=>	=>	End after 7 d repor-ting					
Investigators review e-diary data daily		Start	=>	=>	End		Start	=>	=>	End					
Record AEs, MAAEs, AESI, and SAEs		X	X	X	X	X	X	X	X	X	X	X	X [k]	X [k]	X [k]
Record pregnancies		X	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Record concomitant medication since last visit		X	X	X	X	X	X	X	X	X	X	X	X [l]	X [l]	X [l]
Counsel / remind subjects to perform contraception	X	X	X	X	X	X	X	X	X	X	X	X	X		
Subject hotline availability	Start	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	=>	End
Wellbeing phone call		X [m]					X [m]								

Visit (V)	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
Visit description	Screen-ing	Dose 1 (D1)	1 d after D1	3 d after D1	1 wk after D1	2 wk after D1	Dose 2 (D2)	1 d after D2	3 d after D2	1 wk after D2	2 wk after D2	1 mon after D2	3 mon after D2	6 mon after D2	1 yr after D2 or Early Term. [n]
Visit day/window: - relative to D1	1 to 28 d pre-D1	1	2	3-4	8-10	13-17	29-33								
- relative to D2							1	2	3-4	8-10	13-17	29-33	86-96	171-191	356-376
Total volume of blood drawn per visit	23 mL	134 mL	10 mL	10 mL	9 mL	0 mL	114 mL	10 mL	10 mL	109 mL	15 mL	119 mL	110 mL	95 mL	95 mL

a Brief (symptom-directed) physical examination.

b Vital signs: systolic/diastolic blood pressure, pulse rate, respiratory rate, and oral body temperature. On days with IMP dosing, before and at 1 h (±15 minutes) after dosing.

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

e Viral screen: screen for human immunodeficiency virus (HIV) 1 and 2, Hepatitis B, and Hepatitis C.

f Clinical laboratory tests: chemistry, hematology. Obtain FSH at Visit 0 only if postmenopausal status needs to be confirmed. Troponin will only be measured in the Screening (V0) sample.

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

h VOCBP: The serum beta human chorionic gonadotropin pregnancy test at Visit 0 will be performed using the sample collected for clinical laboratory tests. Before each dosing, urine pregnancy tests will be performed using a commercial kit and the trial subjects will be counseled about the need for the correct use of a highly effective method of contraception.

i If justified by the collected data, the listed blood draw days and the draw volumes may be adapted if not increasing the total number of draw days or the total volume drawn. Leftover blood after completion of the immunogenicity assessments may be used for additional biomarker analyses.

j Trial site personnel will remind the subjects to record the oral body temperature and the worst grade for each symptom in the e-diary at approximately the same time every evening on the day of IMP dosing and then every day in the evening for a total of seven consecutive days. Also, remind the subject to contact the site if they experience any severe or potentially life-threatening reactogenicity events. At V1 assist the subject with downloading the e-diary application, or issue provisioned device; at V9 or Early Termination Visit, collect e-diary or help the subject to delete application.

k Only record SAEs and AESI since last visit.

l Starting with Visit 12, only prohibited concomitant medications will be recorded.

m Trial subjects contacted by phone will be asked non-leading wellbeing questions at >5 h after IMP administration on the day of dosing.

n At the Early Termination Visit, if possible, all assessments planned for the actual week or day of that visit and which are not included in the Early Termination Visit should also be performed.

Notes:

If, for any reason, subjects are permanently discontinued from the trial before completing all scheduled visits, subjects will complete an Early Termination Visit. If possible, all assessments planned for the actual week or day of that visit as listed in the SoA and which are not included in the Early Termination Visit should also be performed. The only exception will be pregnant women, who will not have further research blood draws, but will otherwise complete planned assessments, if required including pregnancy follow-up as described in Section 10.5.3.

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.

As long as the absolute volume of blood drawn does not change per subject overall, the volume of individual blood draws may be adapted if required, e.g., due to unforeseen shortage of specific tubes. Leftover or back up clinical sample aliquots of a subject, which have the exact same collection times, intended to a particular assessment can be used for another assay than that stated on the label if both assessments are described in the SoA and the subject has consented to all. This is only possible if a primary sample has been used first for its original purpose and it is confirmed that no re-testing is required. Samples can only be used interchangeably if the sample characteristics, collection, shipment, and storage conditions are the same.

Abbreviations: AE = adverse event; CMI = cell-mediated immunity/immune response; d = day(s); D = dose; ECG = 12-lead electrocardiogram; Early Term. = early termination; FSH = follicle stimulating hormone; h = hour(s); HLA = human leukocyte antigens; IMP = investigation medicinal product; mon = month(s); MAAE = medically attended adverse event; SAE = serious adverse event; SoA = schedule of activities; V = visit; VOCBP = volunteers of childbearing potential; wk = week(s); yr = year(s).

15.4 Objectives, outcome measures, and endpoints – Substudy D

OBJECTIVES	OUTCOME MEASURES	ENDPOINTS
Primary objectives		
To describe the reactogenicity after one and two doses of the multivalent vaccine candidate BNT166a.	• Proportion (%) of subjects reporting solicited local reactions at the injection site from Dose 1 through Day 7 post-Dose 1 inclusive; and from Dose 2 through Day 7 post-Dose 2 inclusive. • Proportion (%) of subjects reporting solicited systemic events from Dose 1 through Day 7 post-Dose 1 inclusive; and from Dose 2 through Day 7 post-Dose 2 inclusive.	• Solicited local reactions (pain, erythema / redness, induration / swelling). • Solicited systemic events (vomiting, diarrhea, headache, fatigue, myalgia, arthralgia, chills, and fever).
To describe the safety after one and two doses of the multivalent vaccine candidate BNT166a.	• Proportion (%) of subjects with at least one unsolicited AE occurring from Dose 1 through Day 28 post-Dose 1 inclusive. • Proportion (%) of subjects with at least one unsolicited AE occurring from Dose 2 through Day 28 post-Dose 2 inclusive. • Proportion (%) of subjects with at least one SAE occurring from Dose 1 through Day 201 post-Dose 1 inclusive. • Proportion (%) of subjects with at least one AESI occurring from Dose 1 through Day 201 post-Dose 1 inclusive.	• Unsolicited AEs, SAEs, and AESIs recorded by the investigator.
Exploratory objectives		
To describe the neutralizing antibody responses against vaccinia and mpox viruses elicited by the multivalent vaccine candidate BNT166a.	<u>At 2 weeks after Dose 2:</u> • Geometric mean titers. • Geometric means fold rise from baseline (pre-Dose 1). • Proportion (%) of subjects with seroresponse [a].	• Vaccinia-specific neutralizing antibody levels. • MPXV-specific neutralizing antibody levels.
To describe the kinetics and specificities of humoral immune responses elicited by the multivalent vaccine candidate BNT166a.	<u>At all assessed timepoints:</u> • Geometric mean titers.	• Vaccinia-specific neutralizing antibody levels. • MPXV-specific neutralizing antibody levels. • BNT166a antigen-specific binding antibody levels.

a. Seroresponse is defined as seropositivity after baseline for subjects who were seronegative at baseline or two-fold or more increase of the antibody titer compared to the baseline titer for subjects with a pre-existing antibody titer (Pittman et al. 2019). For details, see the statistical analysis plan.

Abbreviations: AE = adverse event; AESI = adverse event of special interest; d = day; IMP = investigational medicinal product; mpox = monkeypox; MPXV = mpox virus; SAE = serious AE.

15.5 Trial population – Substudy D

15.5.1 Selection of trial population

The trial population will be healthy subjects with no prior history of known or suspected smallpox or mpox vaccination (vaccinia-naïve subjects) or Orthopoxvirus infection.

15.5.2 Rationale for trial population

The selection of healthy adult male and female subjects for prophylactic vaccine clinical trials is standard practice to minimize the potential risks which might exist in the subjects with comorbidities and also in subjects taking concomitant medications or who need additional interventions for the underlying disease.

The subjects, who have not received any Orthopoxvirus vaccine and have not been infected by any Orthopoxvirus in the past, will take part in Substudy D to investigate the safety and immune response in this group, representing the majority of the world population who did not have Orthopoxvirus vaccination and exposure.

15.5.3 Inclusion criteria

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

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

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

3 Are 18 through 45 years of age (inclusive) at the time of informed consent.

4 Have a body mass index over 18.5 kg/m^2 and under 30 kg/m^2 and weigh at least 50 kg at Visit 0.

5 Are healthy, in the clinical judgment of the investigator based on volunteer-reported medical history data, physical examination, 12-lead ECG, vital signs, and clinical laboratory test results.

6 Have no prior history of known or suspected smallpox vaccination and no detectable smallpox vaccination characteristic scar (vaccinia-naïve subjects).

7 Agree not to enroll in another trial with an IMP starting from Visit 0 and until the end of this trial (Visit 14).

Virology

8 Negative HIV-1 and HIV-2 antigen/antibody blood test result at Visit 0.

9 Negative Hepatitis B surface antigen and negative core antibodies test results and negative anti-HCV, or negative HCV PCR test result if the anti-HCV is positive at Visit 0.

Reproductive status and contraception

10 VOCBP that have a negative serum 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 (for definitions of postmenopausal and permanently sterilized, see Section 10.5.1).

11 VOCBP who have used a highly effective form of contraception for at least 28 d prior to Dose 1 and agree to practice this method of contraception continuously for 90 d after Dose 2. For guidance on contraception see Section 10.5.2).

12 VOCBP who agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction during trial, starting at Visit 0 and continuously for 90 d after Dose 2.

13 Men who are sexually active with partners of childbearing potential and who have not had a vasectomy that agree to practice a highly effective form of contraception with their sexual partners born female during the trial, starting at Visit 0 and continuously for 90 d after Dose 2 (for guidance on contraception, see Section 10.5.2).

14 Men who are willing to refrain from sperm donation, starting at Visit 0 and continuously for 90 d after Dose 2.

15.5.4 Exclusion criteria

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

1 History of mpox, smallpox or vaccinia infection based on volunteer-reported medical history.

2 Pregnant, breastfeeding, planning pregnancy or planning to father children starting from Visit 0 and continuously until 90 d after receiving Dose 2.

3 History of known or suspected severe adverse reaction including allergic reaction (e.g., anaphylaxis) to vaccines or to vaccine components such as lipids.

4 Current or history of the following medical conditions at Visit 0 or Visit 1:

a) Uncontrolled, moderate or severe asthma; asthma severity as defined in the US National Asthma Education and Prevention Program Expert Panel report, e.g., exclude volunteers who:

- Use a short-acting rescue inhaler (typically a beta 2 agonist) daily, or
- Use high dose inhaled corticosteroids (per American Academy of Allergy Asthma & Immunology), or
- In the past year where any of the following apply:
  - Greater than one exacerbation of symptoms requiring oral/parenteral corticosteroids treatment.
  - Needed hospitalization or intubation for asthma.

b) Chronic obstructive pulmonary disease.

c) Diabetes mellitus type 1 or type 2, including cases controlled with diet alone (Not excluded: history of isolated gestational diabetes).

d) Hypertension: If a person has hypertension, 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.

e) Systolic blood pressure ≥150 mm Hg or diastolic blood pressure ≥100 mm Hg.

f) Malignancy, excluding localized basal or squamous cell cancer.

g) Cardiovascular diseases, (e.g., myocarditis, pericarditis, coronary heart disease, myocardial infarction, congestive heart failure, cardiomyopathy or clinically significant arrhythmias, stroke or transient ischemic attack).

h) Bleeding disorders (e.g., factor deficiency, coagulopathy, or platelet disorder).

i) Seizure disorder: History of seizure(s) within past 3 years; use of medications in order to prevent or treat seizure(s) at any time within the past 3 years.

j) Estimated glomerular filtration rate <60 mL/min/1.73m^2.

k) Chronic liver disease.

5 Schizophrenia, major depressive disorder, suicidal ideation. Such psychiatric illnesses, as bipolar disorder, 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.

6 Current or history of the following diseases associated with immune dysregulation:

- Known or suspected immunodeficiency.
- History of solid organ or bone marrow transplantation.
- Asplenia: any condition resulting in the absence of a functional spleen.
- Currently existing or history of any autoimmune disease.

7 At Visit 0, any screening hematology and/or blood chemistry laboratory value (according to the FDA toxicity grading scale) that meets the definition of a Grade ≥2 abnormality; individuals with clinically non-significant Grade 1 abnormalities (for definition of clinical significance see Section 10.4.1.3.2) may be considered eligible at the discretion of the investigator. Individuals with abnormal but clinically non-significant parameters not included in the FDA toxicity guidance may be considered eligible at the discretion of the investigator. See separate exclusion criteria for bilirubin and troponin I.

8 Abnormal total bilirubin at Visit 0. Note: inclusion of volunteers with bilirubin ≤1.25 ULN if due to Gilbert's syndrome is allowed.

9 Any abnormal troponin I value at Visit 0.

10 A 12-lead ECG at Visit 0 which is consistent with probable or possible myocarditis/pericarditis or which demonstrates clinically relevant abnormalities that may affect subject safety or are otherwise clinically significant findings (e.g., complete left bundle branch block, AV block, average QTcF interval >450 msec, signs of myocardial infarction, ST elevation consistent with myocardial ischemia, or serious brady- or tachyarrhythmias).

11 Febrile illness (body temperature ≥38.0°C) or other acute illness within 48 h prior to Dose 1 and/or current (if presented at Visit 1, temporary deferral is allowed).

12 Participation or planned participation in strenuous or endurance exercise (see Section 5.5 for a definition) within 7 d before or after each IMP administration.

Prior/concomitant therapy

13 Vaccination with any Orthopoxvirus-based vaccine including vaccines for prevention of smallpox, disease caused by vaccinia virus or mpox, or vector Orthopoxvirus-based vaccines.

14 Any vaccination within 28 d before Dose 1. Seasonal inactivated influenza vaccine is allowed, however, it should be administered at least 14 d before IMP administration.

15 Any non-trial IMP within 28 d or five half-lives (whichever is longer) before Dose 1.

16 Blood/plasma products and/or immunoglobulins within 120 d before Dose 1.

17 Allergy treatment with antigen injections within 14 d before Dose 1.

18 Immunosuppressive therapy, including corticosteroids, or radiotherapy within 6 months or five half-lives (whichever is longer) before Dose 1. If systemic corticosteroids have been administered short term (≤14 d, at a dose of ≤20 mg/d of prednisone or equivalent) for treatment of an acute illness, individuals should be enrolled in the trial only after corticosteroid therapy has been discontinued for at least 28 d before Dose 1. Intraarticular, intrabursal, or topical (skin or eyes) corticosteroids are permitted.

Additional exclusions

19 Have a history of alcohol abuse within 1 year before Visit 0 or have a history of substance abuse within the past 5 years before Visit 0.

20 Are vulnerable individuals as per 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.

15.6 Trial treatment – Substudy D

Use	Investigational
IMP name	Multivalent vaccine candidate BNT166a
Type	RNA-based mpox vaccine. The multivalent vaccine candidate includes four RNA components formulated as LNPs. The multivalent DP contains LNP-encapsulated RNAs encoding mpox virus proteins (antigens). Each RNA is purified single-stranded, 5'-capped N1-methylpseudouridine modified ("nucleoside-modified") RNA produced by *in vitro* transcription from a DNA template. The mpox antigens targeted by the multivalent DP are B6, A35, M1, and H3.
Dose level	60 µg [a]
Dosing route and regimen	Two intramuscular doses of the multivalent vaccine candidate, one at Visit 1 (Day 1) and one at Visit 6 (Day 31). Injections should be in the deltoid muscle, using the same non-dominant arm for all IMP doses.
IMP preparation	Trial sites will be provided with the vaccine candidate. Preparation of the IMP will be performed at the trial site following instructions provided in the trial-specific Pharmacy Manual.
Source	BioNTech

a The dose was selected based on Substudy A and Substudy B data.

Abbreviations: DNA = deoxyribonucleic acid; DP = drug product; IMP = investigational medicinal product; LNP = lipid nanoparticle; mpox = monkeypox; RNA = ribonucleic acid.

All provided IMP will be labeled as required per country requirements (see the Pharmacy Manual for details).

15.6.1 Justification for the dose level – Substudy D

The proposed dose level for the multivalent vaccine candidate BNT166a in Substudy D was selected after evaluation of all available 1 month post-Dose 2 safety (including symptom-directed physical examination, vital signs, 12-lead ECG, and clinical laboratory data), reactogenicity, and immunogenicity data from Substudies A and B.

15.7 Statistical considerations – Substudy D

See Section 9.