

CLINICAL TRIAL PROTOCOL

Trial number: BNT331-01

Document version: 2.0 **Document date:** 13 AUG 2024

Sponsor name: BioNTech SE

Sponsor address: An der Goldgrube 12,
55131 Mainz, Germany

Trial title: A Phase I randomized, double-blind, placebo-controlled, safety, tolerability, and pharmacokinetic trial of BNT331 administered in single ascending doses in healthy women and multiple ascending doses in women diagnosed with bacterial vaginosis

Brief lay title: A first-in-human safety trial of BNT331 administered as single ascending doses in healthy women and as multiple ascending doses in women diagnosed with bacterial vaginosis

Trial phase: Ia/b

Indication: Acute and recurrent bacterial vaginosis

Investigational medicinal product (IMP): BNT331 vaginal inserts

Sponsor signatory: Dr Markus Heep, Director Clinical Development, BioNTech SE

Regulatory identifier(s): National Clinical Trial (NCT) number: 06469164; Investigational New Drug application (IND) number: 163436

Trial sites: Sites are planned in the United States (US)

Trial Medical Monitor: For the name and contact details, see the Trial Contact List filed in the Investigator's Site File (ISF)

The "sponsor signatory" is person authorized to sign (approve) the protocol and any protocol amendment(s) for the sponsor. A list of key sponsor personnel involved in the preparation of this protocol and the conduct of the trial, including their full names, titles, roles, and responsibilities, will be maintained. These personnel include the sponsor's medical expert for the trial.

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

Document history	Document date	Document version
First sponsor-approved version	24 NOV 2023	1.0
Second sponsor-approved version	13 AUG 2024	2.0

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

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PROTOCOL UPDATE – SUMMARY OF CHANGES

Overall update rationale

This amendment (from version 1.0 to 2.0) implements changes in the schedule of activities (SoA), inclusion criteria, trial treatment adherence, future research analyses, and introduction to the IMP (as requested by the United States Food and Drug Administration [US FDA]).

Detailed description of changes

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

Section	Reason for change
1.1 and 4.1	Part A: Clarification on admission of subjects from Day -1 until Visit 2 to facilitate timely dosing, post-dose procedures, and sampling.
1.1 and 3	Deletion of detailed analysis from primary objective (Part A and B) and secondary objective (Part B), only endpoints are listed. Addition of anti-drug antibody (ADA) response timepoints i.e., Early Response and Follow-up Visits to secondary objective (Part A and B).
1.1 and 3	Clarification on the timepoints for primary, secondary and exploratory endpoints.
1.1	Deletion of exploratory endpoint analysis from “Statistical Analysis”.
1.1 and 3	Deletion of two exploratory endpoints of Part B, i.e., “BV treatment success and recurrence”.
1.3	Part A: Inclusion of “Visit 3” (Day 7 post-dose) in the footnote “h” for blood sampling (PK) as requested by the US FDA.
1.3	Part A and B: Mentioning of qPCR as an investigation after vaginal swab sampling to align with Section 8.3.4.
1.3	Part B: Addition of blood sampling for ADA response at Early Response and Follow-up Visits as requested by the US FDA. Increase of total blood volume by 10 mL.
1.3	Part A: Footnote “m” is updated to reflect exact day of investigation.
1.3, 4.1 and 6.7	Part A: Clarification on trial treatment administration (Part A) in a lying position.
2.2.3	Additional information on the drug product safety as requested by the US FDA.
5.3.1	Clarification on the duration of abstinence before trial start due to the extended screening period for healthy volunteers in Part A.
6.6.2.1	The blinding status of laboratory personnel is updated to “blinded” to align with the blind management plan.
6.6.2.2	Clarification on blinding language to allow selected IRC members to review unblinded data.
6.9.2	Addition of Centers for Disease Control and Prevention (CDC) treatment guidelines for Bacterial Vaginosis as reference for standard of care treatment options.
7	Addition of “Individual” and “Overall” trial treatment pausing rules.

Section	Reason for change
7.1.3	Deletion of "Related SAE" criteria from permanent discontinuation of trial treatment. Related SAE will be processed as suspected unexpected serious adverse reaction.
8.9	More details on leftover samples for assay qualification or validation are included in "Future research analyses" section.
10.1.4	Clarification on the text for "(S)AE reporting after withdrawal of subject (from trial and/or data processing)" based on the latest Clinical Trial Protocol (CTP) template.
10.1.14	Addition of a new section "Reporting of serious breach" based on the latest CTP template.

1 PROTOCOL SUMMARY

1.1 Synopsis

Trial title

A Phase I randomized, double-blind, placebo-controlled, safety, tolerability, and pharmacokinetic trial of BNT331 administered in single ascending doses in healthy women and multiple ascending doses in women diagnosed with bacterial vaginosis.

Trial rationale

BNT331 active substance is a modified phage-derived endolysin which selectively lyses *Gardnerella spp.* but not other naturally occurring non-pathogenic bacteria in the vagina.

Bacterial vaginosis (BV) is a common vaginal infection, affecting up to 30% of all women worldwide, and has been associated with reproductive and infectious complications. BV is a vaginal dysbiosis characterized by a reduction in healthy vaginal *Lactobacillus* species (*spp.*), and an increase in various anaerobic *spp.*, but particularly *Gardnerella vaginalis* (*G. vaginalis* and other *spp.*). A polymicrobial biofilm comprised of BV-associated bacteria (BVAB) forms on the vaginal epithelium during this infection. Symptoms include vaginal discharge, odor, and irritation; range from mild to severe although some women may remain asymptomatic. Despite initial success of currently recommended treatments, a large proportion of women (up to 60% in some studies) will have recurrent BV. Some women also experience persistent symptoms despite appropriate treatment. Challenges with the most commonly used standard of care antibiotics metronidazole (MDZ) and clindamycin (CLI) include adverse effects, microbiological or clinical resistance formation among some BVAB, including *G. vaginalis*, and the inability of these antibiotics to effectively penetrate the layers of the BV biofilm. Therefore, the high efficacy of BNT331 active substance in eliminating typical *Gardnerella spp.* in *in vitro* biofilms as well as its high specificity and resilience to antibiotic resistance formation, makes BNT331 a promising potential alternative for the treatment of BV, especially in women with recurrent and/or persistent infection.

Clinical development of BNT331 will begin with this first-in-human (FIH) clinical trial BNT331-01. BNT331-01 is a dose-escalation trial designed to assess the safety, tolerability, PK, ADAs, and exploratory efficacy of BNT331 active substance formulated as a vaginal insert in healthy volunteers (HVs) and in women diagnosed with BV by Amsel's criteria and by Nugent score.

Objectives and endpoints

OBJECTIVES	ENDPOINTS
Primary objective	
Part A and Part B	
To evaluate the safety and tolerability of BNT331 when administered vaginally as SAD in healthy women and MAD in women diagnosed with BV.	<p>For each cohort of BNT331 group and combined placebo groups:</p> <ul style="list-style-type: none"> • Occurrence of AEs with onset after the first treatment dose and until 7 days post-dose (Part A) and 120 days after the first dose (Part B). • Occurrence of SAEs with onset after the first treatment dose and until 7 days post-dose (Part A) and 120 days after the first dose (Part B).
Secondary objectives	
Part A and Part B	
To determine the systemic PK of BNT331 active substance, including quantification of systemic absorption when administered vaginally as SAD in healthy women and MAD in women diagnosed with BV.	<p>For each cohort:</p> <ul style="list-style-type: none"> • <u>Part A</u>: The serum concentrations of BNT331 active substance at pre-specified timepoints among subjects who received one single administration. • <u>Part B</u>: The serum concentrations of BNT331 active substance at pre-specified timepoints among subjects who received all scheduled administrations.
To determine ADA response in healthy women and in women diagnosed with BV.	<p>For each cohort:</p> <ul style="list-style-type: none"> • ADA prevalence and change of binding titers against BNT331 active substance in blood before trial treatment (pre-dose) and at 7 days post-dose (Part A) and at 6 days after the first dose, 21 to 30 days after the first dose, and 120 days after the first dose (Part B).
Part B only	
To evaluate the proportion of subjects with diagnosed BV who meet the definitions of clinical cure, Nugent score cure, and responder outcome.	<p>For each cohort of BNT331 group and combined placebo groups:</p> <ul style="list-style-type: none"> • Occurrence of Clinical cure defined as normalization of the vaginal discharge, a negative KOH "Whiff" test, and clue cells <20% of the total epithelial cells/high power field on microscopic examination of the vaginal fluid at 6 days after the first dose and 21 to 30 days after the first dose. • Occurrence of Nugent score cure/Microbiological cure defined as score of <4 at 6 days after the first dose and 21 to 30 days after the first dose. • Responder outcome defined as an occurrence of clinical cure with a normal Nugent score (<4) at 6 days after the first dose and 21 to 30 days after the first dose.

OBJECTIVES	ENDPOINTS
Exploratory objectives	
<p><u>For Part A and Part B:</u> To determine the <u>local</u> PK of BNT331 active substance when administered vaginally as SAD in healthy women and MAD in women diagnosed with BV.</p>	<p>For each cohort:</p> <ul style="list-style-type: none"> <u>Part A:</u> The vaginal fluid concentration of BNT331 active substance over time before (pre-dose), and after trial treatment administration (4 h and 24 h) and at 7 days post-dose. <u>Part B:</u> The vaginal fluid concentration of BNT331 active substance over time before (pre-dose), and 8-24 h after the last trial treatment administration.
<p><u>For Part A and Part B:</u> To determine local ADA response when BNT331 is administered as SAD in healthy women and MAD in women diagnosed with BV.</p>	<p>For each cohort:</p> <ul style="list-style-type: none"> Vaginal ADA presence will be detected as S/N ratio against BNT331 before trial treatment (pre-dose) and at 7 days post-dose (Part A) and 21 to 30 days after the first dose (Part B).
<p><u>For Part A and Part B:</u> To evaluate the changes in the vaginal microbiome of subjects following treatment with BNT331.</p>	<p>For each BNT331 group and combined placebo groups from 5 mg cohort and 20 mg cohort in Part A and Part B, respectively:</p> <ul style="list-style-type: none"> Change of relative abundance of bacterial taxa by shotgun metagenomic sequencing (such as identification of BV-associated bacteria including <i>Gardnerella vaginalis</i> and <i>Lactobacillus spp.</i>) from baseline (before treatment) until 7 days post-dose (Part A) or until 120 days after the first dose (Part B). Change of Nugent score.
<p><u>For Part A and Part B:</u> Change in the levels of <i>Gardnerella</i> and <i>Lactobacillus spp.</i> from baseline (before treatment)</p>	<p>For each BNT331 group from 5 mg cohort and 20 mg cohort in Part A; for each BNT331 group and combined placebo groups from 5 mg cohort and 20 mg cohort in Part B:</p> <ul style="list-style-type: none"> Quantitate the levels of <i>Gardnerella</i> and lactobacilli from baseline (before treatment) to 7 days post-dose (Part A) and to 21 to 30 days after the first dose (Part B).
<p><u>For Part B only:</u> To evaluate the long-term treatment outcome of subjects with diagnosed BV who meet the definitions of clinical cure following treatment with BNT331.</p>	<p>For each cohort of BNT331 group and combined placebo groups:</p> <ul style="list-style-type: none"> Occurrence of subjects reporting new BV symptoms and episodes until 120 days after the first dose.
<p><u>For Part B only:</u> To measure the long-term psychosocial outcome of subjects with diagnosed BV.</p>	<p>Patient-reported outcome before and after treatment</p> <p>For each cohort of BNT331 group and combined placebo groups, including subjects after rescue treatment:</p> <ul style="list-style-type: none"> Change over time in scores of SF-36 and CSFQ-F at three timepoints (Baseline, 21 to 30 days after the first dose, and 120 days after the first dose).

Abbreviations: ADA = anti-drug antibody; AEs = adverse events; BV = bacterial vaginosis; CSFQ-F = Changes in Sexual Functioning Questionnaire; h (s)= hours; KOH = potassium hydroxide; PK = pharmacokinetics; MAD = multiple ascending doses; SAD = single ascending doses; SAEs = serious adverse events; SF-36 = Thirty-six-item short form health survey; *spp.* = species; S/N = signal to noise; ToC = Test of Cure.

Overall design

This is a two-part, randomized, double-blind, placebo-controlled trial to assess the safety, tolerability, PK and pharmacodynamics (PD) and efficacy (for Part B) of BNT331 in healthy women (Part A) and in women diagnosed with BV (Part B).

Since BNT331 will be evaluated for the first time in human, the trial uses a staggered, sequential approach for subject dosing.

Part A

In Part A, 24 adult healthy female volunteers, who are non-pregnant, non-breastfeeding and pre-menopausal will be enrolled in the trial to assess the safety, tolerability, and PK of BNT331.

Part A will include single ascending dose (SAD) levels and will assess the safety of BNT331 and describe the incidence of adverse events (AEs) for subjects randomized at a ratio of 3:1 to BNT331 or placebo. Subjects may be admitted as early as Day -1 until Visit 2 on Day 2, to facilitate timely dosing (on Day 1), post-dose procedures, and sampling. Following investigator's instructions, the subjects will self-administer a single dose of BNT331 on Dosing Day (Visit 1). Directly after dosing, subjects need to remain in a lying position for 6 hours (h) to allow serial sampling. [Table 1](#) denotes the cohorts in Part A.

Table 1: Cohorts (Part A)

Cohort	Single dose (mg)	Number of subjects (N = 24)	
		BNT331	Placebo
1	2	6	2
2	5	6	2
3	20	6	2

This trial will be initiated with Cohort 1 (2 mg), where 2 subjects – ensuring that at least one of the subjects is randomized to the BNT331 arm – will be dosed at the same site and will complete at least 24 h of follow-up post-dose (sentinel dosing approach). If the observing investigator considers the tolerability was acceptable and no stopping/pausing rules are met, the remaining 6 subjects of Cohort 1 will be dosed. Once the last subject of Cohort 1 completes the Follow-up Visit (Visit 3) at 7 days (d) post-dose, all available clinical, laboratory, and other relevant data of the eight subjects will be reviewed by an Internal Review Committee (IRC). If the IRC considers the tolerability was acceptable and no stopping/pausing rules were met, dosing will be opened for the next dose level cohort. Subsequent dose level cohorts will dose under the same rules and will follow the same dose-escalation/assessment process.

Similarly, the overall clinical, laboratory, and other relevant data of all subjects in all dose level cohorts of Part A will be reviewed by the IRC once all subjects in Cohort 3 have completed the Follow-up Visit (Visit 3) at 7 d post-dose. If the IRC considers the tolerability was acceptable and no stopping/pausing rules were met, enrollment will be opened for Part B of the trial.

Part B

In Part B, 72 non-pregnant, non-breastfeeding, pre-menopausal adult women with a diagnosis of symptomatic BV (by Amsel's criteria) will be enrolled in the trial to assess the safety, tolerability, PK, PD, and efficacy of BNT331.

Part B will include multiple ascending dose (MAD) levels. Subjects will be randomized at a ratio of 2:1 to BNT331 or placebo. Following investigator's instructions, the subjects will self-administer the BNT331 dose once daily for 5 d consecutively. Subjects will be enrolled into 2 cohorts ([Table 2](#)).

Table 2: Cohorts (Part B)

Cohort	Five doses of (mg)	Number of subjects (N = 72)	
		BNT331	Placebo
4	5	24	12
5	20	24	12

Part B of this trial will be initiated with Cohort 4 (5 mg). Once all 36 subjects have completed Visit 2 (Early Response Visit), all available clinical, laboratory, and other relevant data will be reviewed by the IRC. If the IRC considers the tolerability to be acceptable and no stopping/pausing rules were met, dosing will be opened for the next/last dose level cohort (Cohort 5, 20 mg).

Trial population

This trial will recruit HVs in Part A and BV subjects in Part B, based on medical history, screening laboratory values, and other inclusion/exclusion criteria. Inclusion and exclusion criteria for Part A and Part B are described in Sections [5.3](#) and [5.4](#) respectively.

Duration of all trial periods

The planned trial duration for each subject in Part A is up to 30 d screening, 1 d Treatment Phase and 12 d of follow-up, which amounts to a maximum total trial duration of 43 d per subject.

For Part B, the planned trial duration is typically up to 3 d screening, 5 d Treatment Phase and follow-up up to Day 120 $\pm$ 7 d which amounts to a maximum total trial duration of 130 d per subject.

Number of trial subjects

A total of 96 subjects will be enrolled in this two-part trial.

For Part A, 24 HVs will be randomized 3:1 to BNT331:placebo in 3 dose level cohorts.

For Part B, 72 BV subjects will be randomized 2:1 to BNT331:placebo in 2 dose level cohorts.

Trial treatments

Treatment name	BNT331	Placebo
Treatment type	Investigational	Non-active investigational product
Active substance	A recombinant protein of around 34.3 kDa, belonging to the 1,4-beta-N-acetylmuramidase group	N/A
Dosage form	Insert	Insert
Unit dose	2 mg, 5 mg, and 20 mg active substance /	N/A

Treatment name	BNT331	Placebo
strength	~1 g insert	
Dosage regimen	<p>Part A: SAD in healthy subjects</p> <p>Cohort 1: Single dose of 2 mg BNT331 active substance</p> <p>Cohort 2: Single dose of 5 mg BNT331 active substance</p> <p>Cohort 3: Single dose of 20 mg BNT331 active substance</p> <p><u>Part B: MAD in women diagnosed with BV</u></p> <p>Cohort 4: 5 mg QD BNT331 active substance for 5 consecutive days</p> <p>Cohort 5: 20 mg QD BNT331 active substance for 5 consecutive days</p>	<p>Part A: SAD in healthy subjects</p> <p>Placebo cohort: Single dose of placebo in corresponding treatment dose cohorts</p> <p><u>Part B: MAD in women diagnosed with BV</u></p> <p>Placebo cohort: Single dose of placebo on 5 consecutive days in corresponding treatment dose cohorts</p>
Administration route	Vaginal	Vaginal

Abbreviations: BV = bacterial vaginosis; MAD = multiple ascending dose; N/A = not applicable; QD = once a day; SAD = single ascending dose.

Statistical analyses

The sample size for each cohort is mainly driven by typical small dose-escalation designs for early detection of potential safety and tolerability events, and is considered adequate to support the trial objectives, while minimizing the number of trial subjects exposed to a new IMP.

The number and percentage of subjects with AEs occurring after first treatment dose and throughout the trial will be tabulated by the Medical Dictionary for Regulatory Activities (MedDRA®) system organ class and preferred term (PT) based on all subjects who have received at least one dose of trial IMP.

Descriptive summary statistics for continuous variables will include the number of subjects, arithmetic mean, SD, minimum, median, and maximum.

The secondary objective of the trial is to either confirm absence of systemic absorption (below lower limit of quantification [LLOQ]), or to determine PK (serum concentration) following vaginal administration of BNT331.

The blood samples will be analyzed for the presence of ADAs in Part A and Part B at the timepoints specified in SoAs (Section 1.3).

Analysis of PK endpoints (as appropriate) will be based on all trial subjects who received all protocol-required doses of BNT331 and have at least one measurable PK concentration from available samples.

In Part B, the number and proportion of subjects with clinical cure, Nugent score cure, or meeting the responder outcome will be tabulated based on the subjects in the modified intent-to-treat (mITT) population.

No formal statistical hypotheses will be tested in this trial. All analyses will be exploratory in nature. All analyses will be provided by dose level of BNT331 active substance and (if applicable) for combined placebo, separately for Part A and Part B.

An interim analysis for Part A might be performed once all subjects of Part A have completed their last visit, and while Part B is ongoing.

Interim analysis for Part B will be performed when the last subject has reached to ToC Visit (Visit 3), to analyze all primary and secondary endpoints, including quantification of *Gardnerella* spp. in vaginal fluid, which is an exploratory endpoint. All data available will be included for evaluation of other endpoints. Interim analyses at other timepoints may be prepared to inform further clinical development.

1.2 Schema (graphical representation of the trial)

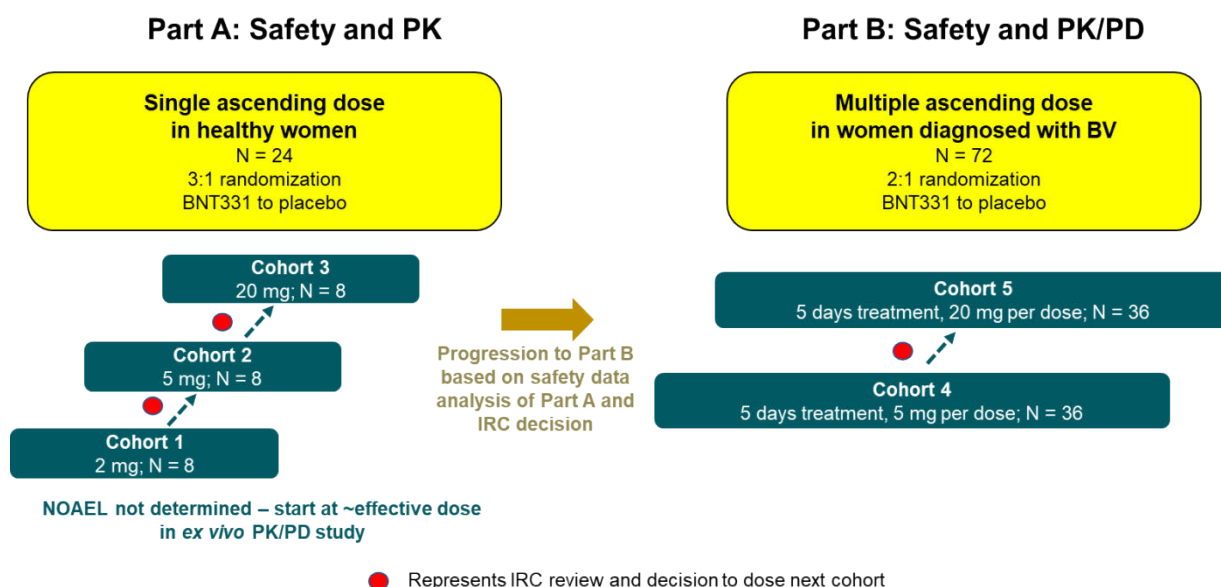


Figure 1: Trial schema

Abbreviations: BV = bacterial vaginosis; IRC = Internal Review Committee; N = total number of subjects in the given cohort; NOAEL = no observed adverse effect level; PD = pharmacodynamics; PK = pharmacokinetics.

1.3 Schedule of activities

The SoAs provide an overview of the trial visits and procedures. The investigator may schedule unplanned visits in addition to those listed in the SoAs, in order to conduct evaluations or assessments required to protect the well-being of the trial subjects.

Table 3: Schedule of activities for Part A

Description	Screening	Dosing	End of therapy	Telephone interview	Follow-up
Visit ID	Visit 0	Visit 1	Visit 2 ⁱ	(No visit)	Visit 3
Time relative to dosing (incl window)	Within 30 d prior Visit 1	NA	1 d post-dose (±0 d)	3 d post-dose (+1 d)	7 d post-dose (+5 d) ^m
Informed consent	X				
Inclusion/exclusion criteria	X	X (confirm)			
Urinalysis ^a	X				X
Pregnancy test and counseling for WOCBP ^b	X	X (pre-dose)			X
Medical history incl. menstrual cycle	X	X (update)			
Demography	X				
Prior and concomitant medications	X	X	X	X	X
Physical examination ^c	X				X
Pelvic examination ^d	X	X (pre- and post-dose)	X		X
Amsel's criteria ^e	X				
Vital signs ^f	X	X (pre- and post-dose)	X		X
Blood draw for clinical laboratory tests ^g (15 mL)	X	X ⁿ (pre-dose)			X
Randomization		X (pre-dose)			
Treatment instructions and dispensing		X (pre-dose)			
Administer trial treatment ^p		X			
Blood sampling for BNT331 active substance concentration (PK) in serum ^h (5 mL)		X (pre-and post-dose)	X		X

Description	Screening	Dosing	End of therapy	Telephone interview	Follow-up
Visit ID	Visit 0	Visit 1	Visit 2 ⁱ	(No visit)	Visit 3
Time relative to dosing (incl window)	Within 30 d prior Visit 1	NA	1 d post-dose (±0 d)	3 d post-dose (+1 d)	7 d post-dose (+5 d) ^m
Blood sampling for ADA response in serum (5 mL)		X (pre-dose)			X
Vaginal swab sampling for local PK levels of BNT331 active substance ^j		X (pre-and post-dose)	X		X
Vaginal swab sampling for ADA response (investigator)		X (pre-dose)			X
Only in subjects in 5 mg and 20 mg DL cohort: Vaginal swab sampling for microbiome assessment ^q		X (pre-dose)			X
Vaginal swab sampling for Nugent score	X ^o	X ^o (pre-dose)			X
Local tolerability assessment (investigator) ^k		X			
Local tolerability interview ^l				X	
Record any AEs and SAEs since last visit	Start	=>	=>	=>	End
Record pregnancies (until 60 d after Visit 3)	Start	=>	=>	=>	=>
Total blood volume collected per visit	~15 mL	~45 mL	~5 mL		~25 mL

- Routine urinalysis by centrally provided dipstick.
- Rapid and highly sensitive urine pregnancy tests for β hCG will be performed using kits provided by sponsor and all subjects will be counseled about the need for highly effective methods of contraception (see Section 10.5.2).
- Complete examination at Visit 0 and a brief (symptom-directed) physical examination at the Follow-up Visit (see Section 8.4.1).
- Pelvic examination (see Section 8.4.8) after 4 h and 24 h, also checking for dissolution of vaginal insert.
- Subjects need to be negative (0/4) for Amsel's criteria (see Section 8.3.2).
- Vital signs: systolic/diastolic blood pressure, pulse rate, and oral body temperature. Height and body weight only at screening (see Section 8.4.2).
- Clinical laboratory (see Section 10.3).
- Before trial treatment, at 1 h (±15 minutes), 2 h (±15 minutes), 4 h (±15 minutes), 6 h (±15 minutes), 24 h (±2 h) after dosing, and at the Follow-up Visit.
- The timing of this visit should be arranged to allow the +24 h (±2 h) PK sampling.
- Before trial treatment, at 4 h (±15 minutes), 24 h (±2 h) after dosing, and at the Follow-up Visit.

- k. Examination of cervix and assessing signs of erythema, oedema, and excoriation on the vaginal mucosa. To be assessed 4 h (± 15 minutes) post-dosing.
- l. A trial site staff will call the subjects and ask about any complaints of dysuria, vulvo-vaginal symptoms such as redness, itching, pain, smell/odor, discharge, and/or bleeding. If during the telephone call, a subject voices genital symptoms, an appointment will be made to see and examine the subject.
- m. Investigations should ideally be performed on 7 d post-dose, while the +5 d time window should only be used in case of specific events such as unexpected onset of menstruation.
- n. If the screening blood sample was taken >3 d before, a new sample will be taken on the day of dosing, results may be pending at start.
- o. Normal Nugent score should be ascertained once at early screening (up to 30 d prior). A new sample will be collected again on the day of dosing, corresponding to the microbiome baseline sample for central analysis (Section [8.3.3](#)).
- p. Directly after dosing, subjects need to remain in a lying position for 6 h to allow serial sampling. Interruptions, such as short walks to the procedure room or the bathroom should be documented as non-important protocol deviations.
- q. Microbiome assessment includes both whole genome sequencing and qPCR.

Note:

The total volume of blood drawn from each trial subject in this trial will be ~90 mL.

Abbreviations: ADA = anti-drug antibody; AE = adverse event; β hCG = beta human chorionic gonadotropin; d = day(s); DL = dose level; h = hour(s); NA = not applicable; PK = pharmacokinetics; qPCR = quantitative polymerase chain reaction; SAE = serious adverse event; WOCBP = women of childbearing potential.

Table 4: Schedule of activities for Part B

Description	Screening	Randomization	Treatment Phase	Early Response (End of Therapy)	Call	ToC	Follow-up/ Recurrence ⁿ or Early termination	Late Follow-up
Visit ID	Visit 0	Visit 1	No visit	Visit 2	No visit	Visit 3 ^l	Visit 4	Visit 5
Day no.	-2 to 1 ^o	-2 to 1 ^p	1 to 5	6 ± 0 ^p	8 to 14	21 to 30	60 ± 7	120 ± 7
Informed consent	X							
Inclusion/exclusion criteria	X	X						
Urinalysis ^a	X			X		X		
Pregnancy test and counseling for WOCBP ^b	X	X						X
Medical history incl. BV and menstrual cycle	X	X (update)						
Demography	X							
Prior and concomitant medications	X	X		X	X	X	X	X
Physical examination ^c	X	X		X		X		
Pelvic examination ^d	X	X		X		X	X	X
Amsel's criteria ^e	X			X		X	X	X
Vaginal swab sampling for Nugent score ^f	X	X		X		X	X	X
Vital signs ^g	X	X		X		X		
Blood draw for clinical laboratory ^h (15 mL)	X			X		X		
Trichomonas rapid test	X							
Vaginal swabs for microbiome analysis		X		X		X	X	X
Treatment instructions and dispensing		X						
Return unused trial treatment				X				

Description	Screening	Randomization	Treatment Phase	Early Response (End of Therapy)	Call	ToC	Follow-up/ Recurrence ⁿ or Early termination	Late Follow-up
Visit ID	Visit 0	Visit 1	No visit	Visit 2	No visit	Visit 3 ^l	Visit 4	Visit 5
Day no.	-2 to 1 ^o	-2 to 1 ^p	1 to 5	6 ± 0 ^p	8 to 14	21 to 30	60 ± 7	120 ± 7
Blood sampling for ADA response in serum (5 mL)		X		X		X	X	
Vaginal swab sampling for ADA response		X				X		
Administer trial treatment ⁱ			X-X-X-X-X					
Blood sampling for BNT331 active substance concentration (PK) in serum ^j (5 mL)		X		X		X		
Vaginal swab sampling for local PK levels of BNT331 active substance ^j		X		X		X		
PROM scores ^m		X				X	X	X
Issue/conclude subject e-diaries ^k		X				X		
Period of daily completion and review of subject e-diaries			Start at Day 1	=>	=>	End		
Record AEs and SAEs since last visit	Start	=>	=>	=>	=>	=>	=>	End
Record pregnancies (until 60 d after Visit 3)	Start	=>	=>	=>	=>	=>	=>	=>
Total blood volume collected per visit	~25 mL			~25 mL		~25 mL	~5 mL	

- Routine urinalysis by centrally provided dipstick.
- Rapid and highly sensitive urine pregnancy tests for β hCG will be performed using kits provided by sponsor and all subjects will be counseled about the need for highly effective method of contraception (see Section 10.5.2).
- Complete examination at Visit 0, and a brief (symptom-directed) physical examination at all later visits (see Section 8.4.1).
- Pelvic examination (see Section 8.4.8), on Day 6 (Visit 2) also checking for dissolution of vaginal insert.
- Amsel's criteria (see Section 8.3.2).

- f. Nugent scoring system (see Section 8.3.3), results may be pending at randomization/dosing.
- g. Vital signs: systolic/diastolic blood pressure, pulse rate, and oral body temperature. Height and body weight only at screening (see Section 8.4.2).
- h. Clinical laboratory tests (see Section 8.4.4), results may be pending at randomization/dosing.
- i. Following Visit 1 (Day 1 until Day 5), treatment will be self-administered by the subject at home, at night before going to bed.
- j. Before trial treatment and at 8-24 h after the last dose (at Early Response and ToC Visit).
- k. Daily recording of treatment adherence (until Day 5), local tolerability/discomfort (until ToC Visit: dysuria, vulvo-vaginal symptoms such as redness, itching, pain, smell/odor, discharge, and/or bleeding).
- l. After the end of the next menstruation, if applicable.
- m. PROM scores, SF-36 and CSFQ-F (see Section 8.4.6).
- n. In case of early termination or BV recurrence at any time after ToC Visit and before the Late Follow-up Visit, the Follow-up/Recurrence Visit assessments should also be performed prior to administration of standard of care treatment and recorded as unscheduled visits, respectively.
- o. Screening and Randomization visit can occur on the same day. In this case, overlapping assessments and procedures as per SoA, may be performed only once.
- p. The first dose of IMP will be inserted at home at bedtime on the day of randomization. Randomization and first dose would be on Day 1. Exceptionally, dosing can be started on one of the next 2 days (within 72 h of screening) at investigators discretion if that allows Visit 2 to fall on a Monday. Randomization would be on Day -1, or Day -2 respectively.
- q. Microbiome assessment includes both whole genome sequencing and qPCR.

Note:

The total volume of blood drawn from each trial subject in this trial will be up to ~80 mL.

Abbreviations: ADA = anti-drug antibody; AE = adverse event; β hCG = beta human chorionic gonadotropin; BV = bacterial vaginosis; CSFQ-F = Changes in Sexual Functioning Questionnaire; d = day(s); h = hour(s); PROM = patient-reported outcome measure; qPCR = quantitative polymerase chain reaction; SAE = serious adverse event; SF-36 = thirty-six-item short form health survey; SoA = schedule of activities; ToC = Test of Cure; WOCBP = women of childbearing potential.

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ABBREVIATIONS/TERMS

Abbreviation	Explanation
~	Approximately
ADA	Anti-drug antibody
AEs	Adverse events
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
BV	Bacterial vaginosis
BVAB	BV-associated bacteria
CDC	Centers for Disease Control and Prevention
CIOMS	Council for International Organizations of Medical Sciences
CLI	Clindamycin
CONSORT	Consolidated Standards of Reporting Trials
C _{max}	Maximum serum concentration
CRO	Contract research organization
CSFQ-F	Changes in Sexual Functioning Questionnaire Form
CSR	Clinical study report
CTFG	Clinical Trial Facilitation Group
D	Day(s)
DS	Drug substance
<i>E. coli</i>	<i>Escherichia coli</i>
eCRF	Electronic case report form
EDC	Electronic data capture (system)
EMA	European Medicines Agency
FDA	Food and Drug Administration
FDAAA	FDA Amendments Act
FIH	First-in-human
FRA	Future research analyses
<i>G. vaginalis</i>	<i>Gardnerella vaginalis</i>
GCP	Good clinical practice
H	Hour(s)
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HVs	Healthy volunteers
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Council on Harmonisation (of Technical Requirements for Pharmaceuticals for Human Use)
ICJME	International Committee of Medical Journal Editors
Ig	Immunoglobulin
IEC	Independent Ethics Committee
IMP	Investigational medicinal product

Abbreviation	Explanation
IND	Investigational New Drug
IPD	Individual trial Participant Data
IRB	Institutional Review Board
IRC	Internal Review Committee
ISF	Investigator's site file
KOH	Potassium hydroxide
LLOQ	Lower limit of quantification
MAD	Multiple ascending dose
MBEC	Minimum biofilm eradication concentration
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDZ	Metronidazole
MED	Minimum effective dose
mITT	Modified intent-to-treat
N/A	Not applicable
PD	Pharmacodynamics
Ph. Eur.	The European Pharmacopoeia
PK	Pharmacokinetics
PROM	Patient-reported outcome measure
PT	Preferred term
QD	Once a day
qPCR	Quantitative polymerase chain reaction
RBC	Red blood cell
RDW	Red cell distribution width
SAD	Single ascending dose
SAEs	Serious adverse events
SAP	Statistical analysis plan
SF-36	Thirty-six-item short form health survey
S/N	Signal to noise
SoA	Schedule of activities
SOPs	Standard operating procedures
<i>Spp.</i>	Species
STI	Sexually transmitted infection
SUSAR	Suspected unexpected serious adverse reaction
$t_{1/2}$	Half-life
$T_{\max}$	Time to $C_{\max}$
ToC	Test of Cure
US	United States (of America)
VMT	Vaginal microbiome transplantation
VVC	Vulvo-vaginal candidiasis
WBC	White blood cell

Abbreviation	Explanation
WOCBP	Women of childbearing potential
Yrs	Years

2 INTRODUCTION

2.1 Trial rationale

BNT331 active substance is a modified phage-derived endolysin which selectively lyses *Gardnerella spp.* but not other naturally occurring non-pathogenic bacteria in the vagina.

BV is a common vaginal infection, affecting up to 30% of all women worldwide, and has been associated with reproductive and infectious complications. BV is a vaginal dysbiosis characterized by a reduction in healthy vaginal *Lactobacillus spp.*, and an increase in various anaerobic *spp.*, but particularly *G. vaginalis* and other *spp.* A polymicrobial biofilm comprised of BVAB forms on the vaginal epithelium during this infection. Symptoms include vaginal discharge, odor, and irritation; range from mild to severe although some women may remain asymptomatic. Despite initial success of currently recommended treatments, a large proportion of women (up to 60% in some studies) will have recurrent BV. Some women also experience persistent symptoms despite appropriate treatment. Challenges with the most commonly used standard of care antibiotics MDZ and CLI include adverse effects, microbiological or clinical resistance formation among some BVAB, including *G. vaginalis*, and the inability of these antibiotics to effectively penetrate the layers of the BV biofilm. Therefore, the high efficacy of BNT331 active substance in eliminating typical *Gardnerella spp.* in *in vitro* biofilms as well as its high specificity and resilience to antibiotic resistance formation, makes BNT331 a promising potential alternative for the treatment of BV, especially in women with recurrent and/or persistent infection.

Clinical development of BNT331 will begin with this FIH clinical trial BNT331-01.

BNT331-01 is a dose-escalation trial designed to assess the safety, tolerability, PK, ADA, and exploratory efficacy of BNT331 active substance formulated as a vaginal insert in HVs and in women diagnosed with BV by Amsel's criteria and by Nugent score.

For the scientific rationale for the trial design, see Section [4.2](#).

For the rationale for the trial treatment, see Section [6.2](#).

2.2 Background

Vaginitis is a common disorder in women of reproductive age, with an estimated prevalence of 20 to 30% worldwide ([Bautista et al. 2016](#)). It encompasses inflammation and/or infection in the vaginal tract, an imbalance in the normal and healthy vaginal microbiota, increased discharge, odor, and/or irritation and is a frequent reason for women to visit their clinical provider. There are different types of vaginitis, however BV primarily affects women of childbearing age ([Reiter and Kellogg Spadt. 2019](#)). BV is more common in women who have multiple sexual partners (of either sex), do not use condoms, douche, and are herpes simplex virus type 2 seropositive. BV is also one of the most prevalent causes of vaginal discharge among women of reproductive age ([Muzny et al. 2019](#)).

Symptoms of a single episode of BV may be mild to severe, but ~25 to 60% of women suffer from recurring BV; and some women experience persistent symptoms despite treatment. Women with recurrent symptomatic BV report a substantial impact on their sexual activity, and overall quality of life and psychological well-being, including reduced self-esteem ([Bilardi et al. 2013](#)). Other major consequences of BV include increased risk of preterm delivery and low birthweight ([Romero et al. 2014](#); [Tabatabaei et al. 2019](#)),

infertility and early spontaneous abortion ([Haahr et al. 2019](#)), and an increased risk for acquisition of HIV and other sexually transmitted infection ([Hoang et al. 2020](#)).

2.2.1 **Pathophysiology and diagnosis of BV**

The optimal vaginal microbiota is characterized by a low diversity and uniform colonization by a few species of *Lactobacillus* ([Vaneechoutte 2017](#)). In BV, an imbalance in the vaginal microbiota occurs with loss of beneficial lactobacilli (*L. crispatus*, *L. jensenii*, *L. gasseri*, henceforth called “c-j-g lactobacilli”) and an overgrowth of facultative and strict anaerobic bacteria BVAB such as *Gardnerella*, *Prevotella*, *Fannyhessea vaginae*, and *Sneathia* ([Ceccarani et al. 2019](#)). However, despite over 60+ yrs of research, the exact etiology of BV remains unknown and continues to be an area of active research.

One current hypothetical model for the etiology of BV postulates that virulent strains of *Gardnerella*, which are present in up to 95% of cases of BV, displace healthy vaginal lactobacilli and form an adherent biofilm on the vaginal epithelium. Synergistic interactions occur with another common BVAB, *Prevotella bivia*. These early interactions facilitate proliferation of other BVAB, normally present in very low numbers, resulting in a polymicrobial biofilm ([Muzny et al. 2019](#)). The innate immune response against this adherent biofilm is weak ([Castro et al. 2018](#)). BV development may be triggered by sexual transmission of a mix of bacteria including *Gardnerella*. It has been suggested that, unlike strictly anaerobic BVAB, *Gardnerella*, a facultative anaerobe, can tolerate the high redox potential created by a *Lactobacillus*-dominated healthy vaginal microbiome ([Muzny et al. 2019](#)). The metabolism of *Gardnerella spp.*, in turn, results in a local increase in pH and a decrease in the redox potential, favoring growth of iron-dependent anaerobes ([Vaneechoutte 2017](#)) and suppression of lactobacilli ([Schwebke et al. 2014](#)).

The clinical diagnosis of BV is based on Amsel’s criteria while a microbiological diagnosis is based on Nugent scoring ([Nugent et al. 1991](#)).

Per the Amsel’s criteria, BV is defined as the presence of at least three of the following four criteria:

- Homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls.
- Vaginal pH >4.5.
(Only for inclusion; *Vaginal pH is not included as a component of the clinical cure for BV.*)
- Positive Whiff-amine test, defined as the presence of a fishy odor when 10% KOH is added to a sample of vaginal discharge.
- Clue cells (i.e., vaginal epithelial cells covered with bacteria) on saline wet mount, comprising at least 20% of epithelial cells per high power field.

Another semi-quantitative microbiologic definition that is frequently used is the Nugent scoring system. Vaginal smears are Gram-stained and scored by the morphology of the observed bacteria (by quantifying gram-positive rods [*Lactobacillus*], gram-negative rods [such as *Gardnerella*, *Prevotella* and *Bacteroides*], and gram-labile rods [*Mobiluncus*]). A Nugent score of 7-10 is indicative of BV, while a score of 4 to 6 is considered intermediate vaginal microbiota and a score of 0 to 3 is considered normal vaginal microbiota.

2.2.2 The medical need

The therapies used currently to treat BV are broad-spectrum antibiotics, mainly MDZ or CLI, antiseptics, probiotics (preparations of lactobacilli), and prebiotics (e.g., lactate gels). Although these treatments may have some beneficial effects, none has proven to have satisfactory efficacy on preventing the development of recurrent BV.

The efficacy of long-term treatment with MDZ among women with recurrent BV is as low as 34% (Sobel et al. 2006). Even in the non-recurrent/uncomplicated form, antibiotics are quite effective in quickly reducing the symptoms, but they are associated with a recurrence rate of up to 60% within 6 months of treatment (Bradshaw and Sobel 2016). Antibiotic treatment not only often fails to eradicate the biofilm but can also lead to further dysbiosis of the vaginal microbiota and can promote the development of VVC (Sobel 2007). Moreover, CLI can trigger *Clostridium difficile* infection, cause antibiotics associated diarrhea and other gastrointestinal side effects (Buffie et al. 2012). Of note, *Gardnerella* strains frequently develop resistance to MDZ (Petrina et al. 2017).

Antiseptic treatments for BV have been less well studied than antibiotics but they are similar with regard to their effectiveness and are also associated with high BV recurrence rates and deterioration of the healthy vaginal microbiota (Swidsinski et al. 2015). Studies of probiotics and prebiotics are generally inconclusive as they appear not to result in lasting benefits (Bradshaw and Sobel 2016). In a previous clinical trial, Lactin-V, a vaginally applied probiotic, was studied when administered after a course of standard of care antibiotics in women with recurrent BV and had only a minor beneficial impact after 10 weeks of therapy (cure rate of 61% vs. 46% for the placebo group) (Cohen et al. 2020).

Various non-pharmaceutical products (e.g., acids, disinfectants) have also been tested as a potential therapy for BV. One recent product is Astodrimer gel, which failed to show a significant improvement in the cure rates for recurrent BV (Schwebke et al. 2021). Apart from the limited success in clinical trials, non-pharmaceutical products tend to be broad-acting and thereby harm the beneficial microbiota at similar rates to the standard of care antibiotics.

VMT after a course of antibiotic treatment had a promising effect in one trial with 5 subjects but relied on successful antimicrobial treatment prior to the transplantation (Lev-Sagie et al. 2019). VMT also face several other challenges, e.g., women with a healthy vaginal microbiota only have a small amount of donor material which poses a problem of scalability for the millions of women suffering from recurrent BV. Furthermore, VMT could increase the risk of transplanting drug-resistant bacteria.

In conclusion, novel treatments that offer an alternative to antibiotics or that can be used in combination with antibiotics, are urgently needed.

2.2.3 Introduction to the IMP

Bacteriophages that infect *Gardnerella* spp. have not been identified yet, however prophages encoding for endolysins are present in the *Gardnerella* genome.

BNT331 active substance consists of 308 amino acids (34.3 kDa) with an N-terminal hydrolytic domain (1,4-beta-N-acetylmuramidase) of the GH-25 domain superfamily and a C-terminal binding domain consisting of 2x CW_7 motifs (see Figure 2). BNT331 active substance cleaves peptidoglycan in the *Gardnerella* cell wall resulting in bacteriolysis. Due to this unique mechanism of action, BNT331 active substance demonstrates no *in vitro* cross resistance with other classes of antimicrobial agents.

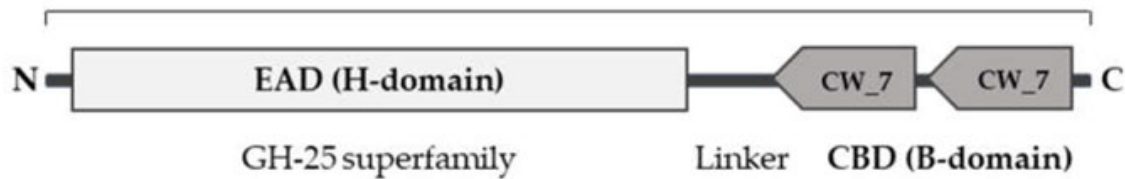


Figure 2: Schematic domain structure of the predicted *Gardnerella* prophage endolysins belonging to the class of 1,4-beta-N-acetylmuramidases

Abbreviations: EAD = enzymatic active domain or H-domain; CBD = cell wall binding domain or B-domain; GH = glycoside hydrolase.

For this agent to be used in clinical development, BNT331 active substance is expressed in *E. coli* and purified to yield an intermediate drug substance of sufficient purity for a non-sterile pharmaceutical preparation. The drug substance is formulated into dry-compressed inserts for vaginal application, using only excipients which comply with the requirements of Ph. Eur. and/or the US Pharmacopeia.

The drug product complies with the requirements of Ph. Eur. and/or the US Pharmacopeia for non-sterile products for pharmaceutical use. Product stability is established according to the ICH.

The BNT331 vaginal insert contains 5% of pure talc (as an excipient) which is US Pharmacopeia-compliant and was tested for absence of asbestos.

The BNT331 vaginal inserts may contain negligible levels of impurities (traces of benzyl chloride and bromobenzyl chloride, raw material leachables), at levels below a limit of detection (20 ng/g), which is 8200-fold below the lifetime acceptable intake level established by [ICH M7 \(R1\) 2023](#) for these impurities.

For more details about BNT331 vaginal inserts, see the current [BNT331 IB](#).

2.3 Benefit/risk assessment

Detailed information about the perceived benefits and potential risks of BNT331 is provided in [BNT331 IB](#).

2.3.1 Benefit assessment

The HVs who participate in Part A of this trial are not expected to benefit from BNT331 administration. However, subjects and others may benefit in the future from information learned from this trial. Through participation in this trial, they may be contributing to the process of developing new therapies for BV.

Subjects in Part B will have access to additional medical evaluations (e.g., trichomoniasis assessments in addition to physical and pelvic examination). Furthermore, participation in this trial could help the trial subjects indirectly, since safety assessments and procedures performed in this trial could uncover previously undiagnosed health problems.

2.3.2 Risk assessment and mitigation strategy

Potential risk source	Summary of data/Rationale for risk	Mitigation strategy
Trial treatment	There is no clinical data on the trial treatment, as this is a first-in-human trial, hence the risk profile is unknown, but could include allergic reactions like vaginal burning sensation, vaginal irritation/ulceration, rash, swelling or anaphylaxis.	<ul style="list-style-type: none"> Subjects with a known history of allergy to components in the IMP will be excluded from the trial. Subjects will be monitored for at least ~6 h (Part A) after the first dose of trial treatment. In an event of Grade 3 or above reaction the subject will be advised to discontinue the trial treatment but will not be discontinued from the trial i.e., will continue with trial visits.
BNT331	The risk of development of ADA exists for all protein-based drugs. The risk of local or systemic ADA-mediated immune reactions is considered low as; a) no prior sensitization is expected, and b) there is no homology to the human proteome.	<ul style="list-style-type: none"> Serum and vaginal fluid samples will be banked, and an initial screening of antibody titers will be assessed using a competition assay. IgG and IgA isotypes will be detected in the assay.
Pregnancy-related risks	Currently information on developmental and reproductive toxicology studies is not available.	<ul style="list-style-type: none"> Pregnant, or seeking to be pregnant women will be excluded from the trial. The investigator or designee will inform the trial subject of the need to use highly effective contraception consistently and correctly. The subject is counseled not to donate or cryopreserve germ cells.
Trial procedures		
Vaginal swab and pelvic examination	Subjects may experience discomfort having the procedures.	<ul style="list-style-type: none"> Trial site personnel is adequately trained to perform both procedures. Trial sites will be selected that are experienced with this type of trial and the procedures described in this protocol.
Blood sampling	During venipuncture, subjects might feel dizzy or faint, or develop a bruise, and/or swelling where the needle is inserted.	<ul style="list-style-type: none"> Venipuncture will only be performed by qualified trial site personnel. The volume of blood drawn at any visit is kept to the amounts required to support the objectives of this trial. Subjects experiencing any adverse effects of a blood draw will be monitored closely by investigator, provided water and/or snacks as needed, and allowed to leave only when their symptoms have resolved.

2.3.3 Overall benefit/risk conclusion

As phages are natural components of human vaginal microbiome ([Abou et al. 2021](#)), phage-derived endolysins are unlikely to have a potential detrimental impact on human reproductive health. To the sponsor's knowledge, there are no known toxic or serious adverse effects reported in the preclinical or a few clinical trials conducted with similar endolysins ([Jun et al. 2017](#)) administered intravenously ([Witzenrath et al. 2009](#)).

BNT331 active substance is a vaginally administered endolysin that is highly specific for *G. vaginalis*. The non-clinical pharmacology and non-clinical safety data obtained to date, summarized in [BNT331 IB](#), indicate the potential for BNT331 to provide clinical benefits to patients with BV, with an acceptable safety profile.

Based on the available information regarding the predicted BNT331 safety profile and the risk mitigation management measures implemented in this FIH trial, the potential risks to the HVs are considered acceptable.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary objective	
Part A and Part B	
To evaluate the safety and tolerability of BNT331 when administered vaginally as SAD in healthy women and MAD in women diagnosed with BV.	<p>For each cohort of BNT331 group and combined placebo groups:</p> <ul style="list-style-type: none"> • Occurrence of AEs with onset after the first treatment dose and until 7 days post-dose (Part A) and 120 days after the first dose (Part B). • Occurrence of SAEs with onset after the first treatment dose and until 7 days post-dose (Part A) and 120 days after the first dose (Part B).
Secondary objectives	
Part A and Part B	
To determine the systemic PK of BNT331 active substance, including quantification of systemic absorption when administered vaginally as SAD in healthy women and MAD in women diagnosed with BV.	<p>For each cohort:</p> <ul style="list-style-type: none"> • <u>Part A</u>: The serum concentrations of BNT331 active substance at pre-specified timepoints among subjects who received one single administration. • <u>Part B</u>: The serum concentrations of BNT331 active substance at pre-specified timepoints among subjects who received all scheduled administrations.
To determine ADA response in healthy women and in women diagnosed with BV.	<p>For each cohort:</p> <ul style="list-style-type: none"> • ADA prevalence and change of binding titers against BNT331 active substance in blood before trial treatment (pre-dose) and at 7 days post-dose (Part A) and at 6 days after the first dose, 21 to 30 days after the first dose, and 120 days after the first dose (Part B).
Part B only	
To evaluate the proportion of subjects with diagnosed BV who meet the definitions of clinical cure, Nugent score cure, and responder outcome.	<p>For each cohort of BNT331 group and combined placebo groups:</p> <ul style="list-style-type: none"> • Occurrence of Clinical cure defined as normalization of the vaginal discharge, a negative KOH "Whiff" test, and clue cells <20% of the total epithelial cells/high power field on microscopic examination of the vaginal fluid at 6 days after the first dose and 21 to 30 days after the first dose. • Occurrence of Nugent score cure/Microbiological cure defined as score of <4 at 6 days after the first dose and 21 to 30 days after the first dose. • Responder outcome defined as an occurrence of clinical cure with a normal Nugent score (<4) at 6 days after the first dose and 21 to 30 days after the first dose.
Exploratory objectives	
<p>For Part A and Part B:</p> <p>To determine the <u>local</u> PK of BNT331 active substance when administered vaginally as SAD in healthy women and MAD in women diagnosed with BV.</p>	<p>For each cohort:</p> <ul style="list-style-type: none"> • <u>Part A</u>: The vaginal fluid concentration of BNT331 active substance over time before (pre-dose), and after trial treatment administration (4 h and 24 h) and at 7 days post-dose. • <u>Part B</u>: The vaginal fluid concentration of BNT331 active substance over time before (pre-dose), and 8-24 h after the last trial treatment administration.

OBJECTIVES	ENDPOINTS
<p><u>For Part A and Part B:</u> To determine local ADA response when BNT331 is administered as SAD in healthy women and MAD in women diagnosed with BV.</p>	<p>For each cohort:</p> <ul style="list-style-type: none"> Vaginal ADA presence will be detected as S/N ratio against BNT331 before trial treatment (pre-dose) and at 7 days post-dose (Part A) and 21 to 30 days after the first dose (Part B).
<p><u>For Part A and Part B:</u> To evaluate the changes in the vaginal microbiome of subjects following treatment with BNT331.</p>	<p>For each BNT331 group and combined placebo groups from 5 mg cohort and 20 mg cohort in Part A and Part B, respectively:</p> <ul style="list-style-type: none"> Change of relative abundance of bacterial taxa by shotgun metagenomic sequencing (such as identification of BV-associated bacteria including <i>Gardnerella vaginalis</i> and <i>Lactobacillus spp.</i>) from baseline (before treatment) until 7 days post-dose (Part A) or until 120 days after the first dose (Part B). Change of Nugent score.
<p><u>For Part A and Part B:</u> Change in the levels of <i>Gardnerella</i> and <i>Lactobacillus spp.</i> from baseline (before treatment).</p>	<p>For each BNT331 group from 5 mg cohort and 20 mg cohort in Part A; for each BNT331 group and combined placebo groups from 5 mg cohort and 20 mg cohort in Part B:</p> <ul style="list-style-type: none"> Quantitate the levels of <i>Gardnerella</i> and lactobacilli from baseline (before treatment) to 7 days post-dose (Part A) and to 21 to 30 days after the first dose (Part B).
<p><u>For Part B only:</u> To evaluate the long-term treatment outcome of subjects with diagnosed BV who meet the definitions of clinical cure following treatment with BNT331.</p>	<p>For each cohort of BNT331 group and combined placebo groups:</p> <ul style="list-style-type: none"> Occurrence of subjects reporting new BV symptoms and episodes until 120 days after the first dose.
<p><u>For Part B only:</u> To measure the long-term psychosocial outcome of subjects with diagnosed BV.</p>	<p>Patient-reported outcome before and after treatment: For each cohort of BNT331 group and combined placebo groups, including subjects after rescue treatment:</p> <ul style="list-style-type: none"> Change over time in scores of SF-36 and CSFQ-F at three timepoints (Baseline, 21 to 30 days after the first dose, and 120 days after the first dose).

4 TRIAL DESIGN

4.1 Overall design

This is a two-part, randomized, double-blind, placebo-controlled trial to assess the safety, tolerability, PK and PD and efficacy (for Part B) of BNT331 in healthy women (Part A) and in women diagnosed with BV (Part B).

Since BNT331 will be evaluated for the first time in human, the trial uses a staggered, sequential approach for subject dosing.

Part A

In Part A, 24 adult healthy female volunteers, who are non-pregnant, non-breastfeeding and pre-menopausal will be enrolled in the trial to assess the safety, tolerability, and PK of BNT331.

Part A will include SAD levels and will assess the safety of BNT331 and describe the incidence of AEs for subjects randomized at a ratio of 3:1 to BNT331 or placebo. Subjects

may be admitted as early as Day -1 until Visit 2 on Day 2, to facilitate timely dosing (on Day 1), post-dose procedures, and sampling. Following investigator's instructions, the subjects will self-administer a single dose of BNT331 on Dosing Day (Visit 1). Directly after dosing, subjects need to remain in a lying position for 6 h to allow serial sampling. [Table 1](#) denotes the cohorts in Part A.

This trial will be initiated with Cohort 1 (2 mg), where 2 subjects – ensuring that at least one of the subjects is randomized to the BNT331 arm – will be dosed at the same site and will complete at least 24 h of follow-up post-dose (sentinel dosing approach). If the observing investigator considers the tolerability was acceptable and no stopping/pausing rules are met, the remaining 6 subjects of Cohort 1 will be dosed. Once the last subject of Cohort 1 completes the Follow-up Visit (Visit 3) at 7 d post-dose, all available clinical, laboratory, and other relevant data of the eight subjects will be reviewed by an IRC. If the IRC considers the tolerability was acceptable and no stopping/pausing rules were met, dosing will be opened for the next dose level cohort. Subsequent dose level cohorts will dose under the same rules and will follow the same dose-escalation/assessment process.

Similarly, the overall clinical, laboratory, and other relevant data of all subjects in all dose level cohorts of Part A will be reviewed by the IRC once all subjects in Cohort 3 have completed the Follow-up Visit (Visit 3) at 7 d post-dose. If the IRC considers the tolerability was acceptable and no stopping/pausing rules were met, enrollment will be opened for Part B of the trial.

Part B

In Part B, 72 non-pregnant, non-breastfeeding, pre-menopausal adult women with a diagnosis of symptomatic BV (by Amsel's criteria) will be enrolled in the trial to assess the safety, tolerability, PK, PD, and efficacy of BNT331.

Part B will include MAD. Subjects will be randomized at a ratio of 2:1 to BNT331 or placebo. Following investigator's instructions, the subjects will self-administer the BNT331 dose once daily for 5 d consecutively. Subjects will be enrolled into two treatment cohorts ([Table 2](#)).

Part B of this trial will be initiated with Cohort 4 (5 mg). Once all 36 subjects have completed Visit 2 (Early Response Visit), all available clinical, laboratory, and other relevant data will be reviewed by the IRC. If the IRC considers the tolerability to be acceptable and no stopping/pausing rules were met, dosing will be opened for the next/last dose level cohort (Cohort 5, 20 mg).

The duration of the trial periods is given in Section [4.1.1](#).

The planned number of trial subjects is given in Section [4.1.2](#).

For the method used for trial subject assignment to trial intervention, see Section [6.6](#).

For standard definitions, e.g., for trial completer and end of trial, see Section [10.6](#).

4.1.1 Duration of the trial periods

The planned trial duration for each subject in Part A is up to 30 d screening, 1 d Treatment Phase and 12 d of follow-up, which amounts to a maximum total trial duration of 43 d per subject.

For Part B, the planned trial duration is typically up to 3 d screening, 5 d Treatment Phase and follow-up up to Day 120 $\pm$ 7 d which amounts to a maximum total trial duration of 130 d per subject.

4.1.2 Planned number of trial subjects

A total of 96 subjects will be enrolled in this two-part trial.

For Part A, 24 HVs will be randomized 3:1 to BNT331:placebo in 3 dose level cohorts.

For Part B, 72 BV subjects will be randomized 2:1 to BNT331:placebo in two dose level cohorts.

4.2 Rationale for the trial design

The number of trial subjects has been chosen to generate adequate safety and PK data to meet the trial objectives, while minimizing the number of trial subjects exposed to the investigational treatment, BNT331.

Double-blind design has been chosen to avoid any bias in the investigator's assessment of treatment response or safety data. The blinding of the sponsor and the CRO will reduce any potential bias in the conduct of the trial and increase the credibility of the efficacy trial results.

Since BNT331 will be evaluated for the first time in humans in this trial, the trial uses a staggered, sequential approach for subject dosing. Pausing/stopping rules will be followed, and there will be ongoing monitoring by a trial IRC that may pause or even permanently discontinue administration of trial treatment, or even permanently terminate the trial at any point in time.

BV is most common among women of childbearing age. Current diagnostic criteria (i.e., Amsel's criteria, Nugent score) are less adequate to diagnose BV in postmenopausal women, as some pathophysiological changes seen in BV overlap with changes seen post-menopause ([Van Gerwen et al. 2023](#)). Additionally, treatment for BV is less standardized in this age group. Due to these considerations, the early phase trials of BNT331 will enroll pre-menopausal women only.

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 written informed consent, see Section [10.1.3](#).

The "end of the trial" date is when the last visit (or procedure) of the last trial subject has occurred in the trial globally.

For guidance on trial discontinuation, see Section [10.1.9](#).

5 TRIAL POPULATION

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

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

5.1 Selection of trial population

This trial will recruit HVs in Part A and BV subjects in Part B, based on medical history, screening laboratory values, and other inclusion/exclusion criteria. Inclusion and exclusion criteria for Part A and Part B are described in Sections 5.3 and 5.4 respectively.

5.2 Rationale for trial population

The rationale for trial population is given in Section 4.2.

5.3 Inclusion criteria

Subjects are eligible for enrollment in this trial only if all the following criteria apply (criteria consider key by the sponsor are highlighted in gray):

5.3.1 Inclusion criteria (Part A)

- 1 Have given written informed consent by signing and dating the ICF before initiation of any trial-specific procedures.
- 2 Subject-reported assigned female sex at birth, at least 18 yrs of age and pre-menopausal, as determined by the investigator.
- 3 Not menstruating or having vaginal bleeding at the Visit 0 and not expecting to menstruate during Visit 1 and until Visit 3.
- 4 Are healthy, in the clinical judgment of the investigator based on volunteer-reported medical history data, physical examination, vital signs, and clinical laboratory test outcomes at Visit 0 (confirmed pre-dose at Visit 1).

Note: HVs with pre-existing stable disease (e.g., obesity, hypertension, etc.), defined as disease not requiring significant change in therapy or hospitalization for worsening disease during the 90 d before Visit 0, can be included.
- 5 Should not have any clinical signs of BV as assessed by the absence of all Amsel's criteria and a normal Nugent score at screening, or other vaginal symptoms, including symptomatic VVC or infection with STI pathogens including *Chlamydia trachomatis*, *Trichomonas vaginalis*, or *Neisseria gonorrhoeae*.
- 6 Able to participate in the trial as an outpatient, to attend all required visits, and to comply with all trial requirements.
- 7 WOCBP must have a negative highly sensitive urine pregnancy test result prior to trial treatment initiation, for definitions of postmenopausal and WOCBP, see Section 10.5.1.
- 8 The subject must have been on the same form of highly effective contraception for at least 3 months prior to dosing (Visit 1) and must agree to keep this method until 60 d after Follow-up Visit (Visit 3), for guidance on contraception, see Section 10.5.2.
- 9 WOCBP who agree not to donate or cryopreserve eggs (ova, oocytes) for the purposes of assisted reproduction during trial, 3 months prior to dosing (Visit 1) and continuously until 60 d after Follow-up Visit (Visit 3).

- 10 Agree to abstain from vaginal intercourse from 72 h prior to dosing until the Follow-up Visit (Visit 3).
- 11 Agree to not use any vaginal products, e.g., creams, gels, foams, sponges, douches, and tampons (except during menstruation) from 72 h prior to dosing until the Follow-up Visit (Visit 3).

5.3.2 **Inclusion criteria (Part B)**

- 1 Have given written informed consent by signing and dating ICF before initiation of any trial-specific procedures.
- 2 Have a clinical diagnosis of BV, defined as having all the following Amsel's criteria (4/4):
 - a) Off-white (milky or gray), thin, homogeneous vaginal discharge.
 - b) Vaginal pH >4.5.
(Only for inclusion; *Vaginal pH is not included as a component of the clinical cure for BV.*)
 - c) Presence of clue cells $\geq 20\%$ of the total epithelial cells/high power field on microscopic examination of the vaginal saline wet mount.
 - d) A positive 10% KOH Whiff test.
- 3 Have a sample collected within 72 h prior to first dose for a Gram stain slide to assess Nugent score by the central laboratory.
- 4 Subject-reported assigned female sex at birth, at least 18 yrs of age and pre-menopausal, as determined by the investigator.
- 5 Not menstruating or having vaginal bleeding at the Visit 0 and Visit 1 and do not expect to menstruate within the next 6 d after Visit 1, until the Early Response Visit (Visit 2).
- 6 Apart from a clinical diagnosis of BV, are healthy, in the clinical judgment of the investigator.

Note: Subjects with pre-existing stable disease (e.g., obesity, hypertension, etc.), defined as disease not requiring significant change in therapy or hospitalization for worsening disease during the 90 d before Visit 0, can be included.
- 7 Able to participate in the trial as an outpatient, to attend all required visits, and to comply with all trial requirements.
- 8 WOCBP must have a negative highly sensitive urine pregnancy test result prior to trial treatment initiation, for definitions of postmenopausal and WOCBP, see Section [10.5.1](#).

- 9 The subject must have been on the same form of highly effective contraception for at least 3 months prior to dosing (Visit 1) and must agree to keep this method until at least 60 d after ToC Visit (Visit 3), for guidance on contraception, see Section 10.5.2.
- 10 WOCBP who agree not to donate or cryopreserve eggs (ova, oocytes) for the purposes of assisted reproduction during trial, starting at Visit 0 and continuously until the Late Follow-up Visit (Visit 5).
- 11 Agree to abstain from vaginal intercourse for the duration of treatment (~5 d) and until ToC Visit (Visit 3).
- 12 Agree to not use any vaginal products, e.g., creams, gels, foams, sponges, douches, and tampons (except during menstruation), until ToC Visit (Visit 3).

5.4 Exclusion criteria

Subjects are not eligible for enrollment in this trial if any of the following criteria apply (criteria consider key by the sponsor are highlighted in gray):

5.4.1 Exclusion criteria (Part A)

- 1 Pregnant, lactating, or planning to become pregnant during their trial participation and for at least 60 d after Follow-up Visit (Visit 3).
- 2 Have active STI, genital lesions, including active herpes simplex virus or syphilitic lesions, or other vaginal or vulvar conditions which could confound the interpretation of the safety and PK results.
- 3 Had received antifungal or antimicrobial therapy (systemic or topical) within 14 d prior to the Visit 1.
- 4 Are using a Copper intrauterine device, or any vaginal hormonal products (including NuvaRing®) as a form of contraception.
- 5 Had a history of drug or alcohol abuse within the past 12 months, as determined by the investigator.
- 6 Had participated in any investigational trial within 30 d before the Visit 1 or is currently participating or plans to participate in any investigational, or observational trial.
- 7 Has any history of allergies, hypersensitivities, or intolerance to the trial treatments including any excipients thereof. See Section 6.1 for list of excipients.
- 8 Has any history of an abnormal Pap smear which required cervical biopsy and/or cervical cauterization within 6 months of Visit 1.
- 9 Malignancy within 5 yrs of screening, including but not limited to cervical carcinoma and carcinomas of the vagina and vulva.
- 10 Has any condition including psychiatric illnesses that could interfere with their ability to understand or comply with the requirements of the trial as determined by

the investigator.

- 11 Vulnerable individuals, i.e., are individuals whose willingness to participate 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. This includes all sponsor, trial site, or third party (e.g., CRO, vendor) personnel directly involved in the conduct of the trial and their family members or dependents, as well as all trial site personnel otherwise supervised by the investigator.

5.4.2 Exclusion criteria (Part B)

- 1 Pregnant, lactating, or planning to become pregnant during trial participation and for at least 60 d after ToC Visit (Visit 3).
- 2 Currently suspected clinically (or confirmed diagnostically) of having alternative causes of vaginal disease symptoms including symptomatic VVC or infection with STI including *Chlamydia trachomatis*, *Trichomonas vaginalis*, or *Neisseria gonorrhoeae*.
- 3 Have active genital lesions, including active herpes simplex virus or syphilitic lesions, or other vaginal or vulvar conditions which could confound the interpretation of the clinical response, as determined by the investigator.
- 4 Had received any antifungal or antimicrobial therapy (systemic or vaginal) within 14 d prior to Visit 1.
- 5 Are using a copper intrauterine device, or any vaginal hormonal products (including NuvaRing®) as a form of contraception.
- 6 Had a history of drug or alcohol abuse within the past 12 months, as determined by the investigator.
- 7 Had participated in any investigational trial within 30 d before Visit 1 or is currently participating or plans to participate in any other investigational, or observational trial.
- 8 Has history of allergies, hypersensitivities, or intolerance to the trial treatments including any excipients thereof.
- 9 Has history of an abnormal Pap smear which required cervical biopsy and/ or cervical cauterization within 6 months of Visit 1.
- 10 Malignancy within 5 yrs of screening, including but not limited to cervical carcinoma and carcinomas of the vagina and vulva.
- 11 Had any condition including psychiatric illnesses that could interfere with the ability to understand or comply with the requirements of the trial as determined by the investigator.
- 12 Vulnerable individuals, i.e., are individuals whose willingness to participate 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. This includes all sponsor, trial site, or third party (e.g., CRO, vendor) personnel directly involved in the conduct of the trial and their family members or dependents, as well as all trial site personnel otherwise supervised by the investigator.

5.5 Lifestyle considerations

The trial subjects will be required to remain at the site for ~6 h for Part A after IMP administration at Visit 1.

The subjects will be advised not to use any vaginally applied modalities, e.g., creams, gels, foams, sponges, douches, and tampons (except during menstruation), and abstain from vaginal intercourse until the Follow-up Visit (Part A) or ToC Visit (Part B), respectively.

Additionally, all subjects of childbearing potential must use a highly effective method of contraception until 60 d after Follow-up Visit (Visit 3) (Part A) and 60 d after ToC Visit (Visit 3) (Part B) as per Section 8.5.5 and Section 10.5.2.

5.6 Screen failures

Screen failures are defined as trial subjects who consent to participate in the clinical trial but are not subsequently assigned to trial treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failures to meet the CONSORT publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, date of informed consent, screen failure details, eligibility criteria, and any SAEs.

Individuals who do not meet the criteria for participation in this trial (screen failures) may be rescreened, advise from medical monitor should be obtained. Rescreened trial subjects must re consent and a new identification number must be assigned.

5.7 Criteria for temporarily delaying enrollment

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

6 TRIAL TREATMENTS AND CONCOMITANT THERAPY

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

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

Concomitant therapy is not considered trial treatment.

6.1 Description of the trial treatments

Treatment name	BNT331	Placebo
Treatment type	Investigational	Non-active investigational product
Active substance	A recombinant protein of 34.3 kDa, belonging to the 1,4-beta-N-acetylmuramidase group	N/A
Dosage form	Insert	Insert
Unit dose strength	2 mg, 5 mg, and 20 mg active substance / ~1 g insert	N/A
Dosage regimen	<u>Part A: SAD in healthy subjects</u> Cohort 1: Single dose of 2 mg BNT331 active substance Cohort 2: Single dose of 5 mg BNT331 active substance Cohort 3: Single dose of 20 mg BNT331 active substance <u>Part B: MAD in women diagnosed with BV</u> Cohort 4: 5 mg QD BNT331 active substance for 5 consecutive days Cohort 5: 20 mg QD BNT331 active substance for 5 consecutive days	<u>Part A: SAD in healthy subjects</u> Placebo cohort: Single dose of placebo in corresponding treatment dose cohorts <u>Part B: MAD in women diagnosed with BV</u> Placebo cohort: Single dose of placebo on 5 consecutive days in corresponding treatment dose cohorts
Administration route	Vaginal	Vaginal
Excipients	Carbomer homopolymer type B Microcrystalline cellulose D-Mannitol Sodium chloride Hydroxyethyl cellulose Talc Magnesium stearate <i>Excipients including D-Mannitol, 2-hydroxypropyl-β-cyclodextrin, and sodium acetate trihydrate are part of BNT331 DS and are present in the final drug product.</i>	Microcrystalline cellulose D-Mannitol Sodium chloride Hydroxyethyl cellulose Talc Magnesium stearate
Packaging and labeling	IMP will be provided in blisters. Blisters will be labeled as required per country requirements.	Placebo will be provided in blisters. Blisters will be labeled as required per country requirements.

6.2 Rationale for the trial treatment

The dose range tested in this trial was chosen based on considerations on the number of required administrations, the maximum tolerable dose, the minimum effective dose, and the maximum manufacturable dose. Without detectable systemic absorption and exposure in sheep and rodent models, except in rabbit (as detailed below), it is unlikely that there will be safety-relevant systemic drug levels achieved in humans with the current BNT331 product and vaginal route of administration. Detailed information about the preclinical studies on BNT331 is provided in the [BNT331 IB](#).

Safety aspects of potential systemic exposure: In a PK study in non-infected sheep with a single vaginal administration of 10 mg BNT331 active substance formulated as tablet, the concentration in plasma remained below LLOQ at any timepoint measured (1 h, 2 h, 4 h, 8 h, and 24 h). Similarly, in a pivotal Good Laboratory Practice-compliant 2-week repeated-dose toxicity study with daily intra-vaginal BNT331 administration (0.1, 0.3 and 1 mg active substance/animal) in female rats, no measurable concentrations (above the LLOQ of 2.5 ng/mL) of BNT331 active substance were detected by a validated method in rat plasma samples collected at multiple timepoints. Average BNT331 active substance levels in rabbit plasma samples collected between 1 h and 6 h post-BNT331 administration on Day 5 were determined according to the qualified method and were in a range of 5 to 26 ng/mL. *In vitro* tolerability experiments on human cell lines have been conducted with 500 µg/mL of BNT331 active substance, such systemic concentrations are unlikely to be reached with the maximal dose tested in this trial.

MED: BV is associated with *Gardnerella spp.* playing a key role in the development of the BV biofilm on the vaginal epithelium. The MBEC is a relevant target for the minimal effective dose. MBEC of BNT331 active substance on 48 to 72 h pre-formed *Gardnerella* biofilms was found to be in the range of 2 to 32 µg/mL across a panel of nine strains (24 h of treatment). The $t_{1/2}$ of BNT331 active substance in human vaginal fluid was on average 14 h. The median C_{max} in sheep vaginal fluid upon single administration of 10 mg tablets was ~3300 µg/mL with T_{max} of 2 h. The MED was derived from a single compartment, zero order PK model based on area under the concentration-time curve as the driver for efficacy. The proposed starting dose for the FIH trial of BNT331 active substance is 2 mg/tablet with a predicted 6 to 8-fold margin over the MED. For the higher dose cohort in the SAD and MAD parts of the clinical trial, 5 and 20 mg doses were chosen to provide additional margin over the MED.

Number of administrations: The goal of the treatment is to eradicate, or massively reduce, the population of *Gardnerella spp.* in the vaginal canal, while giving beneficial lactobacilli sufficient time to recover, re-populate the niche and stabilize the vaginal microbiome, for example, by reducing its pH. A single administration of this investigational treatment is unlikely to achieve this goal, for three reasons: first, lactobacilli grow rather slowly, and *in vitro* data have shown that they need at least 48 h to reach relevant concentration levels (e.g., in minimum inhibitory concentration experiments). Secondly, the half-life of BNT331 active substance is 12 to 15 h in human vaginal fluid, so it is possible that with an imperfect local distribution of BNT331 active substance in the vaginal canal, reservoirs of *Gardnerella spp.* stay viable until the concentration of BNT331 active substance is too low to further reduce them. Third, other intra-vaginal treatments against BV, such as MDZ, require repeated administration. Based on data regarding other vaginal administrations, 5 to 7 doses are typical, and the optimal regimen will have to be determined in the future. This FIH trial will assess the safety, tolerability, PK and PD of five daily doses as a conservative and practical regimen.

6.3 Dosing and administration

BNT331 is a vaginal insert that will be self-administered by the subjects either manually, or using an applicator, which will be sourced by the sponsor and dispensed by the site.

In Part A, BNT331 inserts will be self-administered at the trial site.

In Part B, for optimal exposure, all BNT331 inserts will generally be self-administered at home, at night before going to bed.

Further information on packaging and guidance on administration of IMP, refer to the IMP Manual.

6.3.1 Dose modifications

Dose modifications are not allowed.

6.4 Actions in case of overdose or errors in drug administration

The sponsor does not recommend specific treatment for an overdose. In case of an overdose, further dosing should not be administered.

An overdose is the accidental or intentional use of a drug in an amount (per administration or cumulatively) higher than the dose being studied (for the trial treatment) or higher than the maximum recommended dose according to the authorized product information (for approved concomitant medications).

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

- Contact the sponsor's 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 as well as the duration of the overdose in the eCRF.

The sponsor requires investigators to report/capture events of overdose or errors in drug administration with associated adverse reactions as related AEs. Seriousness and grade should correspond to the associated AE.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the sponsor's medical monitor based on the clinical evaluation of the trial subject.

For more information on error in drug administration, refer to IMP Manual.

6.5 Preparation/handling/storage/accountability

Preparation of trial treatment is not required.

The investigator or designee must confirm that appropriate temperature conditions as per the IMP Manual have been maintained during transit to the trial site and during storage at site for all trial treatment received and any discrepancies are reported and resolved before use of the trial treatment.

In Part A of this trial, subjects will self-administer the (single dose) trial treatment on-site during Visit 1 (Dosing) following the investigator's instructions.

In Part B, subjects will receive detailed instructions from the investigator on how to self-administer the vaginal inserts at home, before going to bed at night.

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 investigator and authorized site personnel until it is handed to the trial subject.

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

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

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

6.6 Subject randomization and blinding

6.6.1 Randomization

Subjects will be randomized at 3:1 for BNT331:placebo in Part A and 2:1 for BNT331:placebo in Part B. Randomization will be implemented using an online randomization tool.

6.6.2 Blinding

In this double-blind trial, all trial site personnel, including the investigator, investigator's staff, and subjects, will remain blinded with regard to trial treatment allocation until the final database lock.

The access to the randomization code will be kept restricted until its release for analysis.

6.6.2.1 Blinding of laboratory personnel

In this double-blind trial, laboratory personnel performing PK, ADA, microbiome, Nugent scoring and safety assessments (see Section 8.6) will remain blinded.

6.6.2.2 Blinding of the sponsor and CRO personnel

Sponsor personnel who routinely have contact with site personnel, CRO personnel, involved in the trial conduct or with any other interest in this double-blind trial will remain blinded with regard to the trial treatment allocation until a data extract is performed after the last subject in Part B has reached ToC Visit (Visit 3) for interim analysis.

The data of Part A might be unblinded prior to this timepoint for an interim analysis on Part A data once all subjects of Part A have completed their last visit.

In Part A and Part B, selected IRC members may be unblinded for the safety review.

During the trial a limited number of BioNTech Bioanalytical personnel and/or CRO personnel may be unblinded to individual subject treatment assignments for the purposes of sample shipment, data review and analysis of PD and PK/PD or any other data. Further details will be specified in a Blind Management Plan.

6.6.2.3 Unblinding

In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a trial subject's trial treatment assignment is warranted. 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 sponsor prior to unblinding a trial subject's treatment assignment unless this could delay further management of the trial subject. If a subject's trial treatment assignment is unblinded, the sponsor must be notified within 24 h after breaking the blind. The unblinded subject will be withdrawn from further trial treatment. The date and reason that the blind was broken must be recorded in the source documentation and eCRF.

For information on permanent discontinuation of trial treatment, see Section [7.1.3](#).

6.7 Trial treatment adherence

After randomization, trial treatment will be dispensed by the investigator or designee and provided to the subject for self-administration. The subjects will be instructed and informed about the correct vaginal administration of the inserts. The dose on Day 1 will be self-administered either on-site (Part A, subjects need to remain in a lying position for 6 h to allow serial sampling, interruptions, such as short walks to the procedure room or the bathroom should be documented as non-important protocol deviations), or at home before going to bed at night (in Part B), for optimal exposure.

In Part A, the date and time of the single dose administered must be recorded in the source documents and in the eCRF.

In Part B, the date and time of dosing will be entered by the subject in their e-diary. The subjects will be asked to return the blister – empty or including any missed doses as applicable – when they return to the site for their next visit.

6.8 Access to trial treatment after the end of the trial

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

6.9 Concomitant therapy

6.9.1 *Prohibited medication during the trial*

Trial subjects must abstain from using any vaginally applied modalities, e.g., creams, gels, foams, sponges, douches, and tampons (except during menstruation), as per Section [5.3](#). Subjects currently taking or planning to take oral or topical antifungal and/or antibacterial treatment cannot be included in the trial. Medically indicated antifungal and antibacterial treatment should not be withheld while on trial treatment. A case-by-case decision with the medical monitor will need to be taken whether subjects can continue trial treatment and/or trial participation.

6.9.2 *Treatment of persistent or recurrent BV*

In case of lack of response (treatment failure) at ToC Visit, subjects will be treated according to local standard of care, expected to fall in the categories as outlined in the CDC treatment guidelines for BV ([CDC Guidelines. 2021](#)).

In case of recurrence of BV after ToC Visit, subjects will receive treatment at any time. However, before standard of care initiation, subjects should have a Recurrence Visit which includes clinical investigations such as Amsel's criteria and microbiological sampling.

6.9.3 Recording concomitant medication during the trial

All concomitant medication permitted or prohibited (including over the counter or prescription medicines, vitamins, and/or herbal supplements) as well as any vaginally applied modalities (e.g., creams, gels, foams, sponges, douches, and tampons [except during menstruation]) that the trial subject receives during the trial must be recorded along with:

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

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

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

7 DISCONTINUATION OF TRIAL TREATMENT AND TRIAL SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation or pausing (temporary halting) of trial treatment

The following pausing rules are established within the trial based on reported AEs, tolerability data, and clinical laboratory results as outlined in Sections 7.1.1 and 7.1.2.

Safety data will be reviewed on ongoing basis by investigators and sponsor.

The sponsor will set up regular IRC meetings (as specified in the IRC charter). In addition, *ad hoc* IRC meetings will be triggered by reported events contributing to pausing rules.

The investigator should notify the sponsor within 24 h about any Grade 3 or higher reaction indicating local intolerance or anaphylaxis reaction as well as SAEs, see Section 10.4.4. In the event of a Grade 3 or higher reaction related to local intolerance or anaphylaxis, the subject will be advised to discontinue the trial treatment but will not be discontinued from the trial, i.e., will continue with trial visits.

If the sponsor confirms reported events fulfill pausing rules criteria:

- The IRC will review all available related data.
- Randomization and trial treatment administration for the impacted dose level cohort will be paused until the IRC recommends that assignment to the cohort and administration of trial treatment may be resumed.

For all subjects who have received trial treatment, all other routine trial conduct activities, including ongoing data entry, reporting of AEs, sample collection, and subject follow-up, will continue during the pause.

7.1.1 Individual trial treatment pausing rules

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

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

7.1.2 Overall trial treatment pausing rules

In Part A and Part B, administration of all trial doses will be paused, pending IRC review:

- For any SAE for which there is no alternative, plausible, attributable cause and which is assessed by the investigator, and/or the sponsor as possibly related.
- Any severe (Grade 3) hypersensitivity or any anaphylactic AE that occurs within 7 d of dosing without a clear alternative cause.
- Any Grade 4 AE after administration of trial treatment that is assessed as related by the investigator, or for which there is no alternative, plausible, attributable cause or any Grade 4 reactogenicity event.

For Grading of AEs and SAEs, refer to Section [10.4.3](#).

7.1.3 Permanent discontinuation of trial treatment

In Part B, trial treatment administration will be permanently discontinued for an individual trial subject if any of the below criteria are met.

- Pregnancy.
- Local intolerance/anaphylactic reaction (Grade 3 or above).

Additional reasons for permanent discontinuation of trial treatment include investigator request, and protocol deviation (including no longer meeting all the inclusion criteria or meeting one or more exclusion criteria).

Trial subjects permanently discontinued from trial treatment will not be discontinued from the trial, i.e., will continue with trial visits (see SoAs Section [1.3](#)).

7.2 Trial subject discontinuation/withdrawal from the trial

In Part B, a trial subject who requests to discontinue receipt of trial treatment, will remain in the trial, and the trial subject should continue to be followed per protocol-specified follow-up procedures. The only exception to this is when a trial subject specifically withdraws consent for further continuation in the trial.

In Part A and Part B, a trial subject may be discontinued/withdrawn from the trial at any time at their own request or at the discretion of the investigator.

Reasons for trial subject discontinuation from the trial include the following:

- Lost to follow-up;
- Death;
- Withdrawal of consent;
- Investigator decision;
- Trial terminated (closure of all sites) by sponsor;

If possible, the Recurrence/Follow-up Visit should be performed. See the SoAs (Section [1.3](#)).

If a trial subject withdraws from the trial, she may request destruction of any samples taken and not tested, and the investigator must document this in the site trial records.

7.2.1 *Withdrawal of consent*

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

7.3 Lost to follow-up

A trial subject will be considered lost to follow-up if they 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 clinic for a required trial visit:

- The 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 the trial subject wishes to and/or should continue in the trial.
- Before a trial subject is deemed lost to follow-up, the investigator or designee must make three individual attempts, spread out over at least a week, to regain contact with the trial subject. These contact attempts should be documented in the trial subject's medical record.
- Should the trial subject continue to be unreachable, they will be considered to have withdrawn from the trial.

7.4 Replacement of permanently discontinued trial subjects

If a subject is discontinued from treatment and/or the trial for reasons other than a safety concern, and if the applicable cohort is still open for randomization, then such subject may be replaced to allow adequate assessment of BNT331 safety, tolerability, and PK.

8 TRIAL ASSESSMENTS AND PROCEDURES

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

All screening evaluations must be completed and reviewed to confirm that potential trial subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects 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 SoAs (Section 1.3) 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 SoAs (Section 1.3), is essential and required for trial conduct, but there may be protocol deviations. Every effort should be made to ensure that protocol-required activities are completed as described. However, it is anticipated that from time to time there may be circumstances outside the control of the investigator that may make it infeasible to perform the planned activity. In these cases, the investigator must take all steps necessary to ensure the safety and well-being 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 as per Laboratory Manual, detailed collection, processing, storage, and shipment instructions and contact information will be provided to the investigator site prior to initiation of the trial.

8.1 Recruitment procedures

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.

8.2 Screening/baseline assessments and procedures

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

- Year of birth
- Age
- Reported assigned sex at birth
- Ethnic group
- Race

During the screening, a complete medical history will be compiled for each trial subject. The history will emphasize the background and progress of the subject's medical condition and include a description of prior therapies for it and the best response achieved by each one. In addition, any ongoing therapies will be recorded.

Procedures conducted as part of the trial subject's routine clinical management (e.g., blood count) and obtained before signing of the informed consent may be utilized for screening or baseline purposes, provided the procedures met the protocol-specified criteria and were performed within the timeframes defined in the SoAs (Section 1.3).

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

Protocol waivers or exemptions from eligibility criteria/the requirements of this protocol are not permitted.

8.3 Inclusion and efficacy assessments

The clinical efficacy will be assessed as secondary endpoint in Part B of the trial (see definition in Section 3) as defined in the [FDA Guidance 2019](#).

8.3.1 **Clinical BV diagnosis/exclusion during screening**

Inclusion criteria will be assessed during screening and confirmed at Visit 1 (see Section 1.3).

In Part A, the Amsel's criteria will be assessed (as per Section 8.3.2) to exclude women with asymptomatic BV. For enrollment qualification, none of the four criteria (0/4) may be met.

In Part B, eligibility for enrollment is confirmed by the presence of all four Amsel's criteria (4/4).

8.3.2 **Assessment of Amsel's criteria**

The Amsel's criteria will be assessed at the visits given in the SoAs (Section 1.3).

Amsel's criteria consist of:

- Homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls.
- Vaginal pH >4.5.
(Only for inclusion; *Vaginal pH is not included as a component of the clinical cure definition for BV.*)
- Positive Whiff-amine test, defined as the presence of a fishy odor when 10% KOH is added to a sample of vaginal discharge.
- Clue cells on saline wet mount, comprising at least 20% of vaginal epithelial cells per high power field.

The examination of the vaginal discharge, vaginal pH, and KOH Whiff test will be performed by a trial site clinician. A vaginal swab sample will be evaluated on-site or sent to the local laboratory to detect and measure the amount of clue cells by saline wet mount microscopy according to the site's routine protocol.

8.3.3 **Nugent scoring**

Nugent scoring will be performed at the visits given in the SoAs (Section 1.3).

A swab sample (and a backup sample) will be collected and processed as per Laboratory Manual and sent to a dedicated specialty bioanalytical laboratory for centralized Nugent scoring.

Vaginal smears are Gram-stained and scored by the morphology of the observed bacteria.

A Nugent score of ≥ 7 at Screening Visit is indicative of BV and required for inclusion in the mITT set (in Part B).

A score of 0 to 3 is considered normal for inclusion in Part A, and would in Part B, if reported at any post-treatment visit, confirm "Nugent score cure"/Microbiological cure as per the secondary endpoints (see Section 3) as defined in the [FDA Guidance 2019](#).

8.3.4 Vaginal swabs for microbiome analysis (Part A [5 mg and 20 mg dose levels cohorts] and Part B)

Vaginal swabs will be collected to assess the microbiome composition by shotgun metagenomic sequencing. Additionally, qPCR will be performed on the vaginal swab samples to quantitate *Gardnerella* and *Lactobacillus* levels. The qPCR and microbiome analysis will be performed at the visits given in the SoAs (Section 1.3).

8.3.5 Telephone interview

In Part A of this trial, a telephone interview will be conducted at 3 to 4 d post-dosing (Visit 1) to assess safety and tolerability of BNT331. If during the phone interview, a subject indicates new or any genital symptoms, an appointment will be made where she can be examined in person, investigator discretion should be applied.

For Part B, trial subjects will be contacted by phone with a non-leading well-being call, refer to Section 1.3.

8.4 Safety assessments

Planned timepoints for all safety assessments are provided in the SoAs (Section 1.3).

8.4.1 Physical examinations

Physical examinations will be performed (by inspection, palpation, and auscultation) by a qualified clinician at the trial site according to the SoAs (Section 1.3).

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

A brief (symptom-directed) physical examination will be performed at the other times noted in the SoAs and as clinically indicated at other timepoints. New or worsened clinically significant abnormalities will be recorded on the AE page of the eCRF.

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

8.4.2 Vital signs, body weight and height

Vital signs (body temperature, pulse rate, and blood pressure) will be assessed by the appropriate trial personnel at the timepoints listed in the SoAs (Section 1.3).

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

Vitals signs measurements should be preceded by at least 5 minutes of rest for the trial subject in a quiet setting without distractions (e.g., television, cell phones).

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

Height and body weight will be measured once and recorded at screening.

8.4.3 Medical history

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

Medical history includes clinically significant diseases, surgeries, previous medical procedures, reproductive status, and all medications (e.g., prescription drugs, over the counter drugs, herbal remedies, and nutritional supplements) used by the trial subject within 14 d prior to treatment start (Day 1).

Additionally, at Screening Visit, menstrual cycle history will be recorded. Menstruation should not be expected in Part A between Visit 1 and Visit 3, or in Part B before Early Response Visit (menstruation is known to interfere with microbiome profile and local PK).

For subjects in Part B, medical history also specifically includes BV history, including number of episodes of BV experienced, treatment used for previous BV, including outcome of previous BV treatment.

Any clinically significant abnormality identified at screening should be recorded as medical history in the eCRF.

8.4.4 Clinical laboratory tests

Planned timepoints for sampling for clinical laboratory tests are provided in the SoAs (Section 1.3). See Section 10.3 for the list of clinical laboratory tests to be performed.

All protocol-required clinical laboratory tests must be conducted in accordance with the central laboratory standard. The investigator must review the laboratory report, assess each out-of-range result as either clinically significant or not clinically significant, and document this review with signature and date.

All laboratory tests with values considered clinically significantly abnormal during participation in the trial should be repeated until the values return to normal/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 notified. Any abnormal laboratory results should be followed up till resolution of the respective laboratory result.

8.4.5 Subject e-diaries (Part B only)

E-diaries will be issued during Visit 1. Subjects will complete their e-diaries from Visit 1 (baseline of self-reported BV symptoms before treatment) until ToC Visit as outlined in the SoAs (Section 1.3).

Subjects will be trained in using e-diaries and will be asked to report treatment adherence, IMP administration issues, tolerability, and evolution of BV signs and symptoms, to gather information regarding dysuria, vulvo-vaginal symptoms such as redness, itching, pain, smell/odor, discharge and/or bleeding. Subjects will also be asked to report on any self-administered vaginal treatment modalities.

If during the ongoing e-diary review, a subject indicates new or worsening genital symptoms, these will be assessed at the next visit or an appointment will be made where she can be examined in person.

8.4.6 Patient-reported outcome measure (PROM) scores (Part B only)

In addition, information will be collected on subject's general well-being and sexual functioning during the trial. The following two PROM scores will be assessed using a tablet-based questionnaire at the timepoints mentioned in the SoA in Table 4:

- SF-36 (Ware and Sherbourne. 1992)

- CSFQ-F ([Clayton et al.1997](#)).

8.4.7 *Trichomonas rapid test (Part B only)*

Trichomonas rapid tests for detection of this parasitic STI will be performed as per local standard of care at the visits given in the [Table 4](#).

8.4.8 *Pelvic examination*

A pelvic examination will be performed to assess local safety/tolerability and to obtain samples at the visits given in the SoAs (Section [1.3](#)).

A qualified clinician will conduct the following pelvic examinations:

- Examination of the external genitalia.
- Speculum insertion for examination of vagina, fornices, and cervix, assessing signs of erythema, petechiae, edema, and abrasion/laceration/excoriation on the vaginal mucosa, dissolution of tablet, or any other relevant findings. Water should be used as the lubricant, as other lubricants can confound the tests to be performed.
- Vaginal swab for pH (at the visits given for Amsel's criteria in the SoAs, Section [1.3](#)).
- Vaginal swab for wet mount (at the visits given for Amsel's criteria in the SoAs, Section [1.3](#)).
- Vaginal swab for Nugent Score (at the visits given in the SoAs, Section [1.3](#)).
- Vaginal swab for PK and ADA (at the visits given in the SoAs, Section [1.3](#)).
- Vaginal swab for microbiome assessments (at the visits given in the SoAs, Section [1.3](#)).

8.5 *Adverse events and SAEs.*

Definitions of AEs and SAEs can be found in Section [10.4](#).

8.5.1 *Time period and frequency for collecting AE and SAE information*

All AE and SAE information will be collected at the timepoints specified in the SoAs (Section [1.3](#)).

AE and SAE collection will continue until the last trial visit.

Medical occurrences meeting the criteria for an SAE (see Section [10.4.2](#)) must be reported as SAEs (see Section [8.5.4](#)).

8.5.2 *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 all directly observed AEs/SAEs as well as all subject-reported AEs/SAEs on the eCRF.

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

The recording, evaluating, and assessing of AEs and SAEs are provided in Section [10.4](#).

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 subject ends trial participation, and they consider the event to be related (see Section 10.4) to the trial treatment or trial participation, the investigator must promptly notify the sponsor as described in Section 10.4.4.

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

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

8.5.3 *Follow-up of AEs and SAEs*

After the initial AE/SAE report, the investigator is required to proactively follow each trial subject at subsequent visits/contacts. All AEs and SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the trial subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Section 10.4.2.2.

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 eCRF.

If a trial subject dies during participation in the trial or during a predefined follow-up period (see Section 1.3), the investigator will provide the sponsor a copy of any postmortem findings including histopathology as soon as it becomes available to the site.

The investigator will submit any updated SAE data to the sponsor within 24 h of receipt of the information.

All ongoing AEs/SAEs will be followed until resolution, considered by the investigator to be stable or chronic (resolved with sequelae), the trial subject is lost to follow-up, or the trial subject withdraws consent. If no final status is reached by the last visit, the investigator must confirm the unavailability of a final status.

8.5.4 *Regulatory reporting requirements for SAEs including SUSARs*

To satisfy regulatory requirements, all SAEs that occur during the trial, regardless of judged relationship to IMP, must be reported to the sponsor by the investigator within 24 h of becoming aware. Completion of the Safety Reporting Form and reporting must not be delayed even if not all the information is available.

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, IRB/IEC, and investigators. The execution of reporting to the different entities may be delegated as detailed in the trial-specific Safety Management Plan.

All serious adverse reactions, the nature, severity, or outcome of which is not consistent with the reference safety information are “unexpected serious adverse reaction”. The expectedness assessment for all related SAEs is based on the reference safety information included in Section 7.1.5 of the [BNT331 IB](#). For the purpose of SUSAR reporting, the version of the IB at the time of occurrence of the SUSAR applies. The sponsor’s Medical Safety & Pharmacovigilance team will determine the expectedness for all SAEs.

All suspected adverse reactions related to the 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.

Information about analysis of similar events will be added to the SUSAR report. IRBs will receive SUSAR reports as applicable.

For the IMP, it is the sponsor’s or delegate’s responsibility to perform expedited SUSAR reporting submission to the applicable regulatory authorities and 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.

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

All SUSARs identified by the sponsor will be reported to the US FDA as IND Safety reports, and all IND Safety reports will be provided to the participating trial investigators or their designee.

If the investigator becomes aware of a related SAE after the end of the trial or receives follow-up information regarding a related SAE, such information should also be reported to the sponsor within 24 h of receipt.

8.5.5 *Pregnancy counseling, testing, and collection of pregnancy information*

At timepoints indicated in the SoAs (Section [1.3](#)), the investigator or designee will inform the trial subject of the need to use the prescribed contraception consistently and correctly, and will document the conversation. This will include counseling linked to the administration of IMPs planned in the trial including their potential effect on fertility and advice about donation and cryopreservation of germ cells for the purposes of assisted reproduction. 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 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.

WOCBP will only be administered trial treatment after negative pregnancy test outcomes at the timepoints indicated in the SoAs (Section [1.3](#)). Additional pregnancy tests may be performed, if necessary, as deemed by the investigator during the trial.

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

For the details about the collection of pregnancy information (see Section 10.5.4).

8.5.6 Death events

Any death that occurs within the observation period will be reported as an SAE, see Section 10.4.2. Date and cause of death will be recorded. If available, a copy of an autopsy report should be submitted if available upon request.

In case of a fatal event, the event term should not be “death” but the underlying event which led to death (death = outcome). If there is more than 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 eCRF needs to be completed.

8.6 Pharmacokinetics

As described in Section 3, the PK parameters will be determined using serum concentrations and vaginal fluid levels of BNT331 active substance. The serum concentrations and vaginal fluid levels of BNT331 active substance will be summarized using descriptive statistics for each cohort in both Part A and Part B.

BNT331 active substance concentration-time profiles will be generated for each cohort in both Part A and Part B.

Remnant clinical sample aliquots of a subject, which have the exact same collection times, intended for a particular testing can be used for another assay than that stated on the label if the testing has been described in the SoAs (Section 1.3) and, the subject has consented to this testing. 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.

8.6.1 Pharmacokinetics – serum BNT331 active substance concentration

Blood sampling for serum BNT331 active substance PK will be performed at the visits and timepoints given in the SoAs (in Section 1.3).

Approximately 5 mL blood per timepoint will be collected, processed and stored according to the Laboratory Manual. The serum samples will be shipped to a specialty bioanalytical laboratory for concentration measurement of BNT331 active substance.

8.6.2 Pharmacokinetics – Vaginal fluid BNT331 active substance levels

Vaginal swab sampling for BNT331 active substance topical PK will be performed at the visits given in the SoAs (in Section 1.3). After pre-dose sample, the first post-dose timepoint (4 h in Part A or 8-24 h after the last dose in Part B) was chosen to minimize interference with local distribution and systemic absorption.

The vaginal swabs intended for measurement of BNT331 active substance levels will be collected, processed, and stored as per Laboratory Manual and will then be transferred to a specialty bioanalytical laboratory for analysis.

8.6.3 Anti-drug antibodies – serum and vaginal fluid

The ADA samples will be collected from all subjects. Planned timepoints for ADA sampling are provided in the SoAs, Section 1.3).

8.7 Pharmacodynamics (Part B only)

The main PD parameter will be the reduction in *Gardnerella spp.*, as described in Section 8.3.4.

8.8 Genetics

Genetic analyses from blood or tissue are not planned in this trial.

Vaginal swabs for microbiome will be analyzed at the timepoints listed in Section 1.3.

Vaginal microbiome analysis by shotgun metagenomic sequencing will be performed by a dedicated specialty bioanalytical laboratory. Sequencing reads will be pre-processed via a database of k-mers and reads of viral and human DNA will be removed digitally at this step and not be made available to the sponsor for analysis. Bacterial reads will be assigned to taxonomic levels based on a maximum likelihood algorithm. After completion of the planned investigations of the microbiome, residual specimens may be used for explorative biomarker research.

If available, the results of microbiome sequencing will be reported in the trial CSR. If data is not available at the time of CSR reporting, then it will be reported separately. All data generated using the genetic samples, will be handled in accordance with applicable laws and regulations; this includes requirements applicable for data protection and a potential withdrawal of consent.

8.9 Future research analyses

Leftover biosamples collected at any time during the trial, e.g., swabs, DNA, serum, samples from consenting trial subjects, may be used for FRA to further characterize biomarkers, e.g., for the development of methods, assay qualification or validation or assay quality controls, and to support further studies to understand mechanism of action of the therapeutic BNT331.

Biosamples for FRA will be retained for use for up to 15 yrs (or shorter if required by local regulations) after the end of this trial. The analysis of data obtained from FRA biosamples is not limited to being analyzed within the clinical trial in which they were collected but can include analysis together with data from other clinical trials.

The availability of a larger dataset will assist in identification and characterization of important biomarkers and pathways to support future drug development.

Biosamples for biobanking for FRA and associated data will be labeled with a unique identification number (pseudonymization) and will not be analyzed on an individual trial subject basis. Data generated using FRA biosamples will not be analyzed on an individual trial subject basis. Thus, the reporting of incidental findings to investigators or subjects is neither possible nor foreseen.

Research performed using FRA biosamples, including all data generated using FRA biosamples, will be handled in accordance with applicable laws and regulations; this

includes requirements applicable for data protection, for sample shipment outside the country of collection, and a potential withdrawal of consent.

For sampling procedures, storage conditions, and shipment instructions, see the Laboratory Manual.

8.10 Blood collection

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

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 trial subject overall, the volume of individual blood draws may be adapted if required, e.g., due to unforeseen shortage of specific tubes.

8.11 Unscheduled visit(s)

An unscheduled visit is any visit of an active trial subject which is not expressly set forth in the SoAs of this protocol, but – as directed by the investigator – is required for the trial or is related to an AE experienced during the trial or may be otherwise required for the health and welfare of the subject.

Such visits may be performed at any time during the trial participation and all assessments performed during such visit must be recorded in the source documents and in the eCRF.

8.12 Treatment failure or recurrence visits (Part B only)

If the examining clinician decides that standard of care treatment for BV is required, the subject will be considered a treatment failure at the ToC Visit.

Recurrent BV after ToC should be investigated in a Recurrence Visit. In this case, all the examinations defined in the SoAs (Section 1.3) for the Recurrence Visit will be performed.

8.13 Early termination visits

If, for any reason, trial subjects are permanently discontinued from the trial before completing all scheduled visits, consenting trial 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 respective SoAs (Section 1.3) and which are not included in the Early Termination Visit should also be performed.

9 STATISTICAL CONSIDERATIONS

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

9.1 Statistical hypotheses

There is no formal statistical hypothesis under test.

9.2 Interim analyses and analysis sequence

An interim analysis for Part B will be performed when the last subject has reached ToC Visit (Visit 3), to analyze all primary and secondary endpoints, including quantification of *Gardnerella spp.* in vaginal fluid which is an exploratory endpoint. All data available will be included for evaluation of other endpoints. Interim analyses at other timepoints may be prepared to inform further clinical development.

9.3 Sample size determination

Planned enrollment is ~96 subjects; 24 in Part A and 72 in Part B, for an adequate evaluation of safety and tolerability. The sample size is mainly driven by typical small dose-escalation designs for early detection of potential safety and tolerability events. With 18 subjects receiving active drug in Part A, the probability of detecting at least 1 AE is 85% if the underlying AE rate is 10%. In Part B, assuming the same AE rate of 10%, with 24 subjects receiving active drug in each treatment arm, the probability of detecting at least 1 AE is 92%.

9.4 Populations for Analyses – Analysis sets

The following analyses sets are defined:

Analysis set	Description
Screened	All trial subjects who sign informed consent
Safety Set	All trial subjects who receive at least a single dose of BNT331 or placebo
Intent-to-treat	All randomized subjects
Modified intent-to-treat	All randomized subjects for Part B, excluding those who subsequently demonstrate a positive test result for other concomitant vaginal or cervical infections at baseline (e.g., <i>C. trachomatis</i> , <i>N. gonorrhoeae</i>), or had any important protocol deviations which may interfere with the efficacy assessment for BV, or who have a baseline Nugent score <7 in central laboratory
PK Analysis Set	All trial subjects who receive all protocol-required doses of BNT331 and have at least one measurable PK concentration from available samples

9.5 Statistical analyses

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

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

9.5.1 General considerations

In general, the statistical analysis will be performed by trial part (i.e., Part A and Part B, respectively) and by dose level and combined placebo.

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

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

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

9.5.2 Primary endpoints

The primary endpoints are defined in Section 3.

All AEs will be coded using the most recent version of MedDRA® coding system.

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

- Any AE
- Related AE
- Grade of AE
- Any SAE
- Related SAE

Moreover, the number and percentage of subjects with any AE will be summarized by worst grade by PT nested within system organ class. All analyses of AEs will be performed on the Safety Set.

9.5.3 Secondary endpoints

The secondary endpoints are defined in Section 3. Analyses based on these endpoints will be further defined in the SAP.

9.5.4 Exploratory endpoints

The exploratory endpoints are defined in Section 3. Exploratory 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 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, European regulation 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, or of applicable laws, 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 yr after completion of the trial.

10.1.3 Informed consent process

Written informed consent must be obtained before any trial-specific procedure is performed.

Trial subjects must be informed that their participation is voluntary.

The investigator or a person designated by 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 international guidelines such as ICH GCP and local regulations (e.g., 21 CFR 50, 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 re-consent.

The investigator or (if applicable) a person designated by the investigator The authorized person obtaining the informed consent must also sign and date the ICF. At least a copy or an original (if required by country regulation) of the ICF(s) must be provided to the trial subject.

Trial subjects must be re-consented to the most current version of the ICF during their participation in the trial, if applicable for the stage of the trial they are in.

10.1.4 Data protection

All data collection and processing during this trial will be performed in accordance with the applicable data protection laws and regulations.

Trial subject's personal data will be stored at the trial site in electronic and/or paper form and will be password protected or secured in a locked file cabinet 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, linking each trial subject's unique identifier to her actual identity and medical record identification.

The investigators must ensure that any trial subject records or datasets that are transferred to the sponsor will contain the identifier only (i.e., will be pseudonymized; 'pseudonymization' means the processing of personal data in such a manner that the personal data can no longer be attributed to a specific data subject without the use of additional information, provided that such additional information is kept separately and is subject to technical and organizational measures to ensure that the personal data are not attributed to an identified or identifiable natural person); 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 on-site and remotely 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 a trial subject withdraws from the trial or is withdrawn from the trial, no new data will be collected, but the sponsor may retain and continue to process data collected before the withdrawal of consent. This includes data processing for the purposes of this trial (as described in this protocol), for related scientific research purposes, for compliance with the legal obligation to establish the safety and efficacy of IMPs, and to support marketing authorization and life-cycle management packages. The latter may include the publishing of IPD (e.g., as required by Phase 2 of the EMA Policy 070). Such published IPD will be anonymized and not contain information which would make the trial subject identifiable. For further details about the disclosure/dissemination of clinical trial data, see Section 10.1.6.

If a trial subject withdraws from the trial, the trial subject may request the destruction of any biosamples taken and not tested. In these cases, the investigator must document this request in the site trial records and the sponsor must ensure the documented destruction of the trial subject's biosamples.

If a trial subject withdraws consent or is withdrawn from the trial, the trial site may contact the subject for follow-up and obtain further health information as needed in case of (S)AEs related to the investigational product(s) if (S)AEs occurred prior to withdrawal of consent and/or 28 d after the last dosing. This information will be shared with the sponsor and regulatory authorities to fulfill 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

An IRC will be established to provide medical and safety oversight of the trial subject safety during the conduct of this trial, with a focus on guidance, management of emergent safety issues, and decision-making as outlined in the IRC charter. It includes periodic in-depth review of blinded safety data by trial subject, cohort, 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 IRC will act as a decision-making body for dose-escalation, and progression from Part A to Part B. The IRC is also a forum for the discussion of other data which could impact the IMP benefit-risk assessment, thereby allowing the IRC to periodically assess the overall benefit-risk of the IMP.

The IRC will be constituted and act according to procedures described in the IRC charter. The IRC will prepare written minutes of its meetings.

10.1.6 Dissemination of clinical trial data

A final [ICH E3](#) conform report integrating all trial results will be prepared by the sponsor as required per applicable laws and regulations. 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.

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.

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.

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 FDAAA. Clinical documents under such laws includes protocols and protocol amendments, SAPs, and ICH E3 CSRs.

If this clinical trial is used to support marketing authorization packages/submissions, the sponsor will comply with the EU Policy 0070, the proactive publication of clinical data on the EMA website. Clinical data, under Phase 1 of this policy, includes clinical overviews, clinical summaries, ICH E3 CSRs, and appendices containing the protocol and protocol amendments, sample eCRFs, and statistical methods. Under Phase 2 of this policy, “clinical data” includes the publishing of individual trial subject data. Such published data will be anonymized and not contain information which would make the trial subject identifiable.

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

The sponsor may provide researchers secure access to IPD, expert summaries, lay summaries, and trial 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 IPD will be anonymized in accordance with applicable privacy laws and regulations. Individual trial subject data will only be shared with researchers in secure environment (e.g., using the Vivli platform) and if the researchers sign suitable confidentiality agreements.

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

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

10.1.7 Data quality assurance

All trial subject data relating to the trial will be recorded on eCRFs 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 signing the eCRF.

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

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 agreed in the Monitoring Plan.

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

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

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

Records and documents, including signed ICFs, pertaining to the conduct of this trial must be retained by the investigator for 25 yrs after trial completion unless local regulations or institutional policies require a longer retention period (e.g., EU law requires archiving for longer if the investigated IMP is marketed). 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 eCRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator should request relevant previous medical records or transfer records, depending on the trial.

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 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). For data entered directly into CRFs, see the CFR completion guidelines.

10.1.9 Early trial site closure and trial termination

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

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

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

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

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

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

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

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

10.1.9.1 Investigator tasks triggered by premature termination of the trial

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

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

10.1.10 Publication policy

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

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

treatment-related information necessary for the appropriate scientific presentation or understanding of the trial results.

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

The sponsor will comply with applicable requirements for publication of trial results. In accordance with standard editorial and ethical practice, including those established by the ICJME. The sponsor will generally support publication of multi-site trials only in their entirety and not as individual site data.

Authorship will be determined by mutual agreement between the sponsor and the investigators and in line with ICJME authorship requirements.

10.1.11 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.

10.1.12 Investigators and trial administrative structure

10.1.12.1 Investigators and trial site personnel

There must be an investigator at each trial site.

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

The responsibilities of investigator(s) must be documented before any trial-related procedure is performed. All persons assigned responsibility as an 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 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 requirements.

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

Curriculum vitae and/or other relevant documents confirming the current qualification of the investigators must be provided to the sponsor. This should include any previous training in the principles of GCP, experience obtained from work with clinical trials, and experience with medical care.

10.1.12.2 Trial site personnel assigned trial-related duties

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

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

Documentation of all involved trial site personnel performing significant trial-related procedures and/or making trial-related decisions must be maintained according to GCP and applicable regulatory requirements, this should include documentation of the tasks assigned, evidence of site personnel training, education and experience.

10.1.12.3 Contract research organizations

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

10.1.12.4 The sponsor and sponsor's personnel

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

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

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

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

10.1.13 Liabilities and insurance

The sponsor is responsible for taking out relevant clinical trial insurance in accordance with local law and regulations.

10.1.14 Reporting of serious breaches

A serious breach is any deviation from the approved protocol version or any applicable regulation (e.g., the [EU Regulation No 536/2014](#)) 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 h after the site becomes aware of the event:

- [REDACTED]

The sponsor will assess each suspected serious breach to decide whether it should be reported as a serious breach. The sponsor will comply with regional/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

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

Data entered manually will be submitted to the sponsor through use of an EDC system, data extracts, and reports. Trial sites will be responsible for 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. eCRFs 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 his/her trial site in a readable format that must be kept with the trial records. Acknowledgment of receipt of the trial subject data will be obtained.

10.2.2 Electronic case report forms

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

All eCRFs should be completed as per the CFR completion guidelines by designated, trained trial site personnel. eCRFs should be reviewed, verified, and then electronically signed and dated by the investigator or a designee.

10.2.3 Subject-reported outcomes

E-diaries and PROM scores will be used in Part B of this trial, see Sections [8.4.5](#) and [8.4.6](#), respectively.

10.2.4 Investigator's site file and the trial master file

The principal investigator is responsible for the filing of all essential documents in an ISF. The sponsor is responsible for the timely filing of all essential documents in the trial master file. 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

The tests listed in Table 5 will be performed by the central laboratory at the time/days listed in Section 1.3.

Table 5: Clinical laboratory test parameters

Laboratory assessments	Parameters		
Hematology	Platelet count	RBC indices: RDW MCV MCHC MCH	WBC count with differential (absolute numbers only): neutrophils, lymphocytes, monocytes, eosinophils, basophils.
	RBC count		
	Hemoglobin		
	Hematocrit		
Blood chemistry	BUN	AST	Total bilirubin
	Creatinine	ALT	Lactate dehydrogenase
	Non-fasting glucose	Alkaline phosphatase	

The routine urinalysis including specific gravity, pH, glucose, total protein, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase will be performed locally by the clinical sites using centrally provided dipsticks.

Additional tests may be performed at any time during the trial as determined necessary by the investigator or required by local regulations.

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

10.4.1 Definition of AE

AE definition:

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

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

10.4.1.1 Events meeting the AE definition:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., radiological scans, vital signs measurements), including those that worsen from Screening Visit, 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.
- New conditions or worsening of pre-existing conditions detected or diagnosed after trial treatment administration.

- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either trial treatment or a concomitant medication.

10.4.1.2 Events not meeting the AE definition:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the trial subject's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the trial subject's condition. However, specific symptoms resulting from the progression and fatal cases due to the progression must be documented as AEs and reported as SAEs if applicable.
- 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 eCRF. 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.

10.4.1.4 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

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

- Results in death
- Is life-threatening:
 - The term “life-threatening” in the definition of “serious” refers to an event in which the trial subject was at risk of death at the time of the event. It does not refer to an event, **which hypothetically might have caused death, if it were more severe.**
- Requires hospitalization or prolongation of existing hospitalization:
 - In general, hospitalization signifies that the trial subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether “hospitalization” occurred or was necessary, the AE should be considered serious.
 - Hospitalization for elective treatment of a pre-existing condition that did not worsen from Screening/Dosing Day Visit 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.
- 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 intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

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

The terms “severe” and “serious” are not synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe, or according to [DAIDS 2017](#)); see 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 eCRF.

SAEs must be reported by the investigator to the sponsor within 24 h after learning of the event; see Section [10.4.4](#) for reporting instructions.

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.

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:

- Hospitalizations for respite care will not be considered as reportable SAE.
- Hospitalizations solely for coordination of care, including hospice arrangements, will not be considered as reportable SAE.
- Hospitalizations that were necessary solely because of trial subject requirement for care outside of normal outpatient clinic operating hours will not be considered as reportable SAE.
- Planned hospitalizations required by the protocol (e.g., for trial treatment administration or insertion of access device for trial treatment administration) will not be considered as reportable SAE.
- Hospitalizations for procedures or interventions of a pre-existing condition of the trial subject (elective surgery = planned, non-emergency surgical procedure) will not be considered as a reportable SAE (unless the intervention/procedure is not caused by an acute worsening of the pre-existing condition during the time trial participation):

- If it was planned and documented in trial subject record before the trial-specific trial subject informed consent was signed (ICF for trial participation, see Section 10.1.3), or
- If it was scheduled during the trial when elective surgery became necessary, and the trial subject has not experienced an AE.

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

- If trial subjects are enrolled with underlying disease, the progression of underlying disease during trial participation is not considered as AE. However, specific symptoms resulting from the progression and fatal cases due to the progression must be documented as AEs and reported as SAEs if applicable.
- 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 8.5.1).

- Data pertaining to AEs will be collected during each trial visit either based on the trial subject's spontaneous description or investigator's inquiry or discovered in the course of examinations done during the visit, clinical significance of any sign or symptom needs to be evaluated by the investigator.
- Clinically significant laboratory findings need to be documented as AEs in the source data and eCRF. 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 eCRF 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 eCRF.
- 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.
- The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE. If a definitive diagnosis is not possible, the individual signs and symptoms should be recorded.

Assessment of severity

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

The intensity of (serious) AEs will be graded by the investigator. Grading should follow the [DAIDS 2017](#). Where specific guidance for an adverse event term is not provided, the following general approach should be followed:

- Grade 1 – Mild; does not interfere with the trial subject's usual function
- Grade 2 – Moderate; interferes to some 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
- Grade 5 – Fatal

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

- 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.

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:

- 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

Investigator assessment of the outcome of an AE

The investigator has to assess the outcome of an AE 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)
- 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 screening is defined as recovered as soon as the medical condition has returned to Screening Visit 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 AE/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 eCRF 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, he/she 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.

In this trial, it cannot be excluded that during the course of the trial some procedures give rise to AEs which are related to the trial procedure and not to the trial treatment.

Procedure-related AEs can occur during or after trial-specific procedure (e.g., discomfort

after blood drawing). These events must be reported in the eCRF on Adverse Event pages as “related to trial procedure” with the causing procedure specified.

Notes applicable for relationship to trial procedures categories

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

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

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

10.4.4 Reporting of SAEs and Grade 4 AEs

All AE and SAE information will be collected at the timepoints specified in the SoAs (Section 1.3).

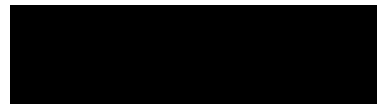
All SAEs and Grade 4 AEs which occur in a trial subject during the observation period, whether considered to be associated with trial medication or not, must be reported by the investigator to the sponsor within 24 h of first knowledge of the event.

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.

Safety 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 as soon as possible.

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

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

For SAEs, follow-up information should be sent to the sponsor (indicating that this is a “follow-up” report using the respective Safety Reporting Form) without delay as described above and accompanied by appropriate anonymized supporting documentation (e.g., discharge letters, medical reports or death certificates), until a final outcome and date are

available. All confidential information (name, address, full day of birth) needs to be redacted before sending.

For SAEs, in addition to a medical record, the investigator should complete an Additional Information and Follow-Up Form, which contains the SAE term and subject number.

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 sponsor's medical monitor for this trial should be contacted.

10.5 Contraceptive guidance and collection of pregnancy information

The following definitions and guidance is based on the CTFG recommendations related to contraception and pregnancy testing in clinical trials issued in 2020 ([CTFG 2020](#)).

10.5.1 Definitions

Women of childbearing potential

For the purpose of this document, a woman is considered of childbearing potential if they are fertile, following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

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

Postmenopausal female

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

For women with suspected onset of menopause, investigator discretion should be applied to determining trial entry.

10.5.2 Contraception guidance

The following guidance describe what is considered highly effective methods of contraception.

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

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

- Combined estrogen and progestogen-based hormonal contraception associated with inhibition of ovulation, i.e., established use of (oral or transdermal) hormonal methods of contraception (vaginal hormonal products such as NuvaRing® are prohibited).

- Progesterone-only based contraception associated with inhibition of ovulation, i.e., established use of (oral, injected, or implanted) hormonal methods of contraception.¹
- Intrauterine hormone-releasing system (copper intrauterine devices are prohibited).¹
- Bilateral tubal occlusion.¹
- Bilateral vasectomy (male partner of a trial subject).^{1, 2}
- Sexual abstinence.³

Note: Intrauterine copper device and any vaginal hormonal methods of contraception are not acceptable for this trial even if considered highly effective.

- 1 Contraception methods that in the context of this guidance are considered to have low user dependency.
- 2 A vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial subject.
- 3 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 Rationale for the level of contraception

Currently there are no data on human teratogenicity/fetotoxicity among the IMP to be used in this trial. Therefore, all trial subjects biologically capable of having children must agree to use a highly effective method of contraception consistently and correctly as specified in the SoAs (Section 1.3).

At timepoints indicated in the SoAs (Section 1.3), the investigator or designee will inform the trial subject of the need to use the contraception consistently and correctly, and will counsel the subject not to donate or cryopreserve germ cells until 60 d after Follow-up Visit (Visit 3) (Part A), and until 60 d after ToC Visit (Visit 3) (Part B), respectively. 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. The investigator or designee will document this conversation at the timepoints indicated in the SoAs (Section 1.3).

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

WOCBP will only be administered trial treatment after negative pregnancy test outcomes at the timepoints indicated in the SoAs (Section 1.3).

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

Given the nature and mechanism of action of BNT331, there is currently no scientific rationale for an interaction or a suspected interaction that could lead to a clinically relevant reduction of the efficacy of hormonal methods of contraception due to BNT331 administration. Therefore, the use of hormonal contraception is allowed in this trial.

10.5.4 Collection of pregnancy information

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication will be reported as an AE or SAE. If a trial subject becomes or is found to be pregnant after trial drug administration, the investigator will report any pregnancy during the AE reporting period specified in Section 1.3 and will follow the pregnancy up to outcome and report any abnormal outcomes as detailed in this protocol. The complete pregnancy as well as the newborn will be followed up for 12 months after delivery and the information will be forwarded to the sponsor.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and to be reported via the Safety Reporting Form (see Section 10.4.4). Any post-trial pregnancy-related SAE considered reasonably related to the trial treatment by the investigator will be reported to the sponsor.

Pregnancy information will only be collected after obtaining written informed consent from the pregnant trial subject.

The initial and follow-up information must be documented on the paper-based Pregnancy Reporting Form and submitted to the sponsor within 24 h of learning of a trial subject's pregnancy. The completed form needs to be sent to the Safety Report Fax number or E-Mail given in Section 10.4.4.

The investigator will request follow-up information on the trial subject and the child until the child's first birthday at least two times, i.e., at the time of expected date of delivery and around the first-year birthday of the child. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, maternal or newborn complications and their presumed relation to the IMP, birth defects, and the presence or absence of any congenital abnormalities and any delay in developmental milestones. In order to collect further follow-up information on the health status of the newborn and its development, both parents (or the parent with medical custody) need to provide written consent. Information to be collected includes the presence or absence of any congenital abnormalities, birth defects, maternal or newborn complications and their presumed relation to the IMP. Generally, the follow-up will be of a duration determined in consultation with the pediatrician.

A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-trial pregnancy-related SAE considered reasonably related to the trial treatment by the investigator will be reported to the sponsor.

The investigator does not need to report subjects/patients who are found to be pregnant between screening and IMP administration, provided no protocol-related procedures were applied.

10.6 Standard definitions

10.6.1 Definition of trial subject

After providing informed consent, volunteers are considered trial subjects.

10.6.2 Definition of trial completer

A trial subject is considered to be a trial completer if they have completed the last visit shown in the SoAs (see Section 1.3).

10.6.3 Definition of enrolled subjects

Trial subjects who receive a dose of IMP are enrolled trial subjects.

10.7 Country-specific requirements

Not applicable.

10.8 Prior protocol amendments and version updates

The protocol amendment summary of changes for the current update is located directly before the protocol summary.

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