CLINICAL STUDY PROTOCOL

A SINGLE-CENTER, OPEN-LABEL STUDY TO INVESTIGATE THE MASS BALANCE, EXCRETION PATHWAYS AND METABOLITES AFTER A SINGLE ORAL DOSE OF 500 MG, 3.7 MBq, [^{14}C]BTZ-043 IN HEALTHY MALE VOLUNTEERS

CONFIDENTIAL

Sponsor code: LMU-IMPH-BTZ-043-03
PRA code: NVS543EC-185431
EudraCT number: 2021-000449-42

Investigational product:	BTZ-043
Clinical phase:	Phase 1 study
Indication to be studied:	Not applicable
Sponsor:	LMU Klinikum Marchioninistr. 15 81377 Munich Germany
Contract Research Organization and Clinical Site:	PRA Health Sciences – Early Development Services Van Swietenlaan 6 9728 NZ Groningen The Netherlands
Principal Investigator:	Jan Jaap van Lier, MD Phone: +31 50 402 2325 Fax: +31 50 402 2223 E-mail: lierjanjaapvan@prahs.com

Version 2.0, 01 Jul 2021
~~**Version 1.0, 18 Mar 2021**~~

This study will be performed in compliance with the principles of Good Clinical Practice.

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SPONSOR AUTHORIZATION OF CLINICAL STUDY PROTOCOL

The Sponsor and the Contract Research Organization agree to conduct the study as outlined in this clinical study protocol. Any modification of the clinical study protocol must be agreed upon by the Sponsor and the Contract Research Organization and must be documented in writing.

Name/Position:	Date:	Signature:
Sponsor: LMU Klinikum		
Michael Hoelscher, MD, FRCP (Lond) Sponsor's Delegated Person	05 Juli 2021	
Piet Swart, PhD, Dip PharMed Sponsor's Scientific Advisor	01 Jul 2021	

AUTHORIZATION OF CLINICAL STUDY PROTOCOL BY CONTRACT RESEARCH ORGANIZATION

The Sponsor and the Contract Research Organization agree to conduct the study as outlined in this clinical study protocol. Any modification of the clinical study protocol must be agreed upon by the Sponsor and the Contract Research Organization and must be documented in writing.

Name/Position: Date: Signature:

Contract Research Organization: PRA Health Sciences – Early Development Services

I recognize that all information concerning this trial is not previously published and is confidential information. This includes the Investigator´s Brochure, trial protocol, case report forms, assay methods, technical methodology, and scientific data.

By my signature below, I hereby certify that I have more than 2 years of experience in the conduct of clinical studies and that I have read, understood, and agree to abide by all conditions, instructions, and restrictions contained in this protocol. I confirm that I was trained in the serious adverse event (SAE) reporting.

Jan Jaap van Lier, MD
Principal Investigator
PRA Group BV, a PRA Health Sciences company

23 July 2021 .....

SERIOUS ADVERSE EVENT CONTACT INFORMATION

In case of a serious adverse event (see Appendix 8.2), the Principal Investigator will send a report within 24 hours of notification to:

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SUMMARY OF CHANGES

The following changes have been introduced in this Version 2.0 of the protocol (dated 01 Jul 2021) and are given as a combination of double underlined and italic text. Deleted text is given as ~~double strikethrough~~.

The rationale for these changes is the change in blood sample volumes and timepoints, and changes in the reporting of metabolite profiling/identification and PK. In addition, contact information of the Client's Clinical Project Manager has been altered.

The changes can be found in:

- *Contact Information*
- *Synopsis*
- *Table 1 - Schedule of Assessments*
- *Section 3.5.1.3 Total Blood Volume: Table 3 - Number and Volume of Blood Samples and Total Blood Volume Collected per Subject*
- *Section 3.5.3.3 Exploratory Variables*
- *Section 3.5.4 Drug Concentration Measurements*
- *Section 3.6.2.1 Pharmacokinetic Evaluation*
- *Section 3.6.2.3 Evaluation of Exploratory Variables*

SYNOPSIS

Study Title

A SINGLE-CENTER, OPEN-LABEL STUDY TO INVESTIGATE MASS BALANCE, EXCRETION PATHWAYS AND METABOLITES AFTER A SINGLE ORAL DOSE OF 500 MG, 3.7 MBq, [^{14}C]BTZ-043 IN HEALTHY MALE VOLUNTEERS

Study Codes

Sponsor code : LMU-IMPH-BTZ-043-03
PRA code : NVS543EC-185431
EudraCT number : 2021-000449-42

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Sponsor's Delegated Person : Michael Hoelscher, MD, FRCP (Lond)
Sponsor's Clinical Project Managers : Julia Dreisbach, VMD and Petra Gross-Demel, PhD

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Promotor's contact : Florian Kloss, PhD

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Client's Clinical Project Manager : ~~Flavia Koch, PhD, MSc~~ *Astrid Patzlaff, PhD*

Contract Research Organization and Clinical Site

PRA Health Sciences – Early Development Services, Van Swietenlaan 6, 9728 NZ Groningen, The Netherlands

Principal Investigator

Jan Jaap van Lier, MD

Objectives

Primary : To determine the rates and routes of excretion of [^{14}C]BTZ-043-related radioactivity, including mass balance of total drug-related radioactivity in urine and feces (and vomit, if applicable), following the oral administration of a single 500 mg dose of [^{14}C]BTZ-043 in healthy male volunteers.
: To determine the pharmacokinetics (PK) of total radioactivity in blood and in plasma.
: To characterize the plasma PK of BTZ-043 and main metabolites by liquid chromatography-mass spectrometry (LC-MS), if applicable.
: To characterize the urine concentrations of BTZ-043 and main metabolites by LC-MS, if applicable.

Secondary : To assess the safety and tolerability of a single 500 mg oral dose of BTZ-043 administered to healthy volunteers.

Exploratory : To identify BTZ-043's metabolites in plasma, and to quantify metabolites not measured in the primary objectives by radiometry in order to elucidate key biotransformation pathways and clearance mechanisms of BTZ-043 in humans.

: To identify BTZ-043's metabolites in excreta (urine and feces) and to quantify metabolites not measured in the primary objectives by radiometry in order to elucidate key biotransformation pathways and clearance mechanisms of BTZ-043 in humans.

Design and Treatments

This study is a Phase 1, single-center, open-label study to investigate the absorption, metabolism, and excretion of BTZ-043 after a single oral administration of 500 mg BTZ-043 containing 3.7 MBq of [^{14}C]BTZ-043 in 4 healthy adult male subjects.

A total of 4 evaluable subjects completing all procedures are required. Six (6) subjects will be enrolled in the cohort in order to have 4 evaluable subjects. If the cohort of 4 subjects administered with 500 mg BTZ-043 containing 3.7 MBq of [^{14}C]BTZ-043 leads to less than 4 evaluable subjects, the 2 back-up subjects can be dosed subsequently to ensure that 4 subjects have completed all study procedures and are evaluable. If any of the 4 subjects vomits within 4 hours after study drug administration (approximately 2 times the median T_{max} of BTZ-043), the subject will be replaced. The 2 back-up subjects will stay in the clinical research center until the morning of Day 2.

The study will consist of a screening period (Day -21 to -2), a baseline period (Day -1), a single dose treatment on Day 1 with a minimum of 96 hours (=4 days) post dose in-house observation period (Days -1 up to afternoon Day 5), and a follow-up visit 30 days (±2 days) after the [^{14}C]BTZ-043 dose. The subjects will be admitted to the clinical site on Day -1, at least 17 hours before the administration of [^{14}C]BTZ-043 for baseline evaluation. Subjects will be administered a single 500 mg [^{14}C]BTZ-043 dose as drinking suspension. Subjects will be confined to the clinical site for at least 96 hours following drug administration (ie, afternoon of Day 5). During this time, blood, feces, and urine samples for measurement of [^{14}C]BTZ-043 and metabolites will be collected. The radioactivity excreted in urine and feces as well as radioactivity in blood and plasma should be measured and the data should be communicated daily to Matthias Bader or his designee. The subjects will be released from the clinic approximately 96 hours to 168 hours after dose administration and upon satisfactory recovery of radioactivity (at least 90%) approved by the Sponsor's scientific advisor after consulation of the Sponsor. This will depend on the radioactivity excretion balance as assessed by a normal counting method or real time rapid analysis on site ('quick counting' procedure, utilizing liquid scintillation counter, or sample combustion apparatus) of radioactivity in plasma, urine, and feces accumulated until 96 hours, as feasible. The data has to be available before subjects will be released from the clinic.

The in-house period should be prolonged up to the morning of Day 8 if at least 1 of the following applies:

- Total radioactivity in plasma is higher than 5% of total radioactivity-C_{max} and/or PK parameters for determination of the total radioactivity-elimination phase cannot be determined reliably.
- The mass balance in urine and feces is not complete by Day 5 (ie, <90%).
- The combined urinary and fecal excretion is not ≤1% of the administered dose per 24 hours for 2 consecutive days based on ^{14}C-radioactivity "quick counts" or normal counts, as feasible.

In the event of an incomplete mass balance on the morning of Day 8, subjects may be discharged from the clinical site upon discretion of the Investigator. Subjects with an incomplete mass balance (<90% of dose administered and/or combined urinary and fecal excretion >1% of the administered dose per 24 hours for 2 consecutive days based on ^{14}C-radioactivity "quick counts" or normal counts, as feasible) on Day 8 will be asked to return to the clinical site for one or two 24-hour visits (Day 11-12 and Day 15-16).

In the event of subjects not defecating for 2 days, measures will be taken to ensure defecation (eg, additional fibers, plum juice, dried prunes, and/or sufficient hydration).

Study Schedule

Screening	: Between Day -21 and Day -2.
Treatment period	: From Day -1 (admission) to approximately 96 hours after study drug administration (Day -1 up to afternoon Day 5) with possible extension (Day 6 to Day 8) as well as possible 24-hour visits (Day 11-12, Day 15-16).
Follow-up	: 30 days (±2 days) after the [^{14}C]BTZ-043 dose.

Subjects

4 healthy male subjects

Main Criteria for Inclusion

Age	: 18 years to 55 years, inclusive, at screening
Weight	: 55 to 90 kg, inclusive, at screening
Body mass index	: 18.0 to 29.0 kg/m^2, inclusive, at screening

Study Drug

Active Medication

Active substance	: BTZ-043
Activity	: Inhibition of *Mycobacterium tuberculosis* DprE1
In development for	: Tuberculosis
Dose	: 1 x 100 mL (equivalent to 500 mg BTZ-043)
Strength	: 5 mg/mL BTZ-043 containing 0.037 MBq/mL of [^{14}C]-BTZ-043
Dosage form	: Oral suspension
Manufacturer	: Prepared by pharmacy at PRA

Variables

Safety	: Adverse events, clinical laboratory, vital signs, 12-lead electrocardiogram, physical examination
Pharmacokinetics	: Total radioactivity concentrations in plasma, whole blood, urine, and feces Total radioactivity in plasma and whole blood PK parameters estimated using noncompartmental analysis, as appropriate : C_{max}, t_{max}, $t_{1/2}$, AUC_{inf}, AUC_{0-t}, and whole blood to plasma ratios Concentrations of BTZ-043 and main metabolites in plasma and urine Plasma and urine BTZ-043 PK parameters estimated using noncompartmental analysis, as appropriate: C_{max}, t_{max}, $t_{1/2}$, k_{el}, AUC_{inf}, AUC_{0-t}, CL/F, V_z/F, Ae_{urine}, Fe_{urine}, and CL_r Plasma and urine metabolites PK parameters estimated using noncompartmental analysis, as appropriate: C_{max}, t_{max}, $t_{1/2}$, k_{el}, AUC_{inf}, AUC_{0-t}, Ae_{urine}, and Fe_{urine} Urine and feces (and vomit, if available) radioactivity PK parameters estimated using noncompartmental analysis, as appropriate: Ae_{urine}, Fe_{urine}, Ae_{feces}, Fe_{feces}, and, if available, Ae_{vomit}, and Fe_{vomit}
Exploratory	: Metabolite profiles, metabolite identification, as feasible $\%AUC_{total\ radioactivity}$ of BTZ-043 and metabolites

Statistical Methods

Safety parameters	:	Descriptive statistics
PK parameters	:	Descriptive statistics for all relevant PK parameters: n, number of missing values to be reported (m), geometric mean, geometric coefficient of variation, mean, coefficient of variation, SD, median, minimum, and maximum
Exploratory parameters	:	Descriptive statistics

Table 1 Schedule of Assessments

Visit	Screening	Treatment Period[a]									24-hour Visits[a]				Follow-up
Study Day	Days -21 to -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6[m]	Day 7[m]	Day 8[m]	Day 11[m]	Day 12[m]	Day 15[m]	Day 16[m]	Day 31 (±2 days)
Confinement[a]		X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Ambulatory	X														X
Admission		X									(X)		(X)		
Discharge[b]							X	(X)	(X)	(X)		(X)		(X)	
Informed Consent	X														
Medical History	X														
Demographics	X														
Physical Examination[c]	X	X													X
Body Weight	X	X													
Height and BMI Calculation	X														
Serology (HBsAg, anti-HCV, anti-HIV 1 and 2)	X														
SARS-CoV-2 PCR[d]		X		X							(X)		(X)		
Drug and Alcohol Screen	X	X													
Clinical Laboratory[e]	X	X		X		X									X
Urinalysis	X	X		X		X									X
12-lead ECG[f]	X	X	X	X			X	(X)	(X)	(X)		(X)		(X)	X
Vital Signs[g]	X	X	X	X			X	(X)	(X)	(X)		(X)		(X)	X
Eligibility Check	X	X	X												
Study Drug Administration			X												
Plasma Sampling for BTZ-043 and Main Metabolites[h]			X	X	X										
Whole Blood Sampling for Radioactivity[i]			X	X	X	X	X	(X)	(X)	(X)	(X)		(X)		
Plasma Sampling for Total Radioactivity and Metabolite Profiling[i]			X	X	X	X	X	(X)	(X)	(X)	(X)		(X)		

Visit	Screening	Treatment Period[a]									24-hour Visits[a]				Follow-up
Study Day	**Days -21 to -2**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6[m]**	**Day 7[m]**	**Day 8[m]**	**Day 11[m]**	**Day 12[m]**	**Day 15[m]**	**Day 16[m]**	**Day 31 (±2 days)**
Urine Collection and Pooling for BTZ-043 and Main Metabolites, Total Radioactivity, and Metabolite Profiling[j]		X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Feces Collection for Total Radioactivity and Metabolite Profiling[k]	X	X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Vomit Collection for Total Radioactivity, if available[l]			X												
Previous and Concomitant Medication	X	X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X
AE Monitoring		X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X

AE=adverse event; BMI=body mass index; ECG=electrocardiogram; HBsAg=hepatitis B surface antigen; HCV=hepatitis C virus; PCR=polymerase chain reaction; PK=pharmacokinetic(s)

a Subjects will be in the clinic from Day -1 (predose) until Day 5 with possible extension (Day 6 to Day 8) as well as possible 24-hour visits (Day 11-12, Day 15-16). The 2 back-up subjects will stay in the clinic until the morning of Day 2.

b Subjects will be released from the clinic 96 hours after dose administration and upon satisfactory recovery of radioactivity.

c Complete physical examinations will be conducted at screening, Day -1, and follow-up/early termination. Symptom driven physical examinations may be conducted at any time, per the Investigator's discretion.

d Sampling of nasal and throat mucosal cells. If deemed necessary, additional tests may be conducted during the study. The nonclinical project manager will be informed immediately upon availability of Day 11 results, as this will qualify the feces samples for metabolite profiling with regard to potential COVID-19 infectivity.

e Clinical laboratory tests (including clinical chemistry, hematology, and urinalysis): at screening; on Day -1 (admission), Day 2, and Day 4; and at follow-up.

f 12-lead ECG: at screening; Day -1, and 1 hour, 4 hours, and 24 hours postdose; at discharge; and at follow-up.

g Vital signs (supine systolic and diastolic blood pressure, pulse, body temperature, and respiratory rate): at screening; at admission; at predose and at 1 hour, 4 hours, and 24 hours postdose; at discharge; and at follow-up.

h Plasma sampling for PK of BTZ-043 and main metabolites in plasma: at predose and 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16, 24, 36, and 48 hours postdose.

i Total radioactivity in whole blood and plasma~~, and metabolite profiling in plasma~~: at predose and 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16, 24, 36, 48, 72, and 96 hours postdose (in case total radioactivity recovered is still <90%: in addition 120, 144, and 168 hours postdose and at further 24-hour visits [264 and 360 hours postdose] until study completion). *Metabolite profiling in plasma: at predose and 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16, 24, 36, 48, 72, and 96 hours postdose (in case total radioactivity recovered is still <90%: in addition 120, 144, and 168 hours postdose.*

j Urine collection and pooling for BTZ-043 and main metabolites, total radioactivity, and metabolite profiling: at predose (within 12 hours prior to dosing) and over 0-24, 24-48, 48-72, and 72-96 hours postdose collection intervals (in case total radioactivity recovered is still <90%, in addition 96-120, 120-144, and 144-168 hours postdose and at further 24-hour intervals [Day 11-12 and Day 15-16] until study completion).

k Feces collection for total radioactivity and metabolite profiling; predose feces collection to be collected at any time within 48 hours prior to study drug administration (at home or in the clinic). After administration of BTZ-043, feces will be collected in 24 hour collection intervals up to 96 hours postdose (in case total radioactivity recovered is still <90%: in addition 120, 144, and 168 hours postdose and at further 24-hour intervals [Day 11-12 and Day 15-16] until study completion).

l If produced, vomit will be collected up to 8 hours after the administration of the study drug for total radioactivity determination.

m Day 6-8 procedures will only be conducted in case that the recovery of radioactivity was not satisfactory until Day 5. 24-hour visits procedures will only be conducted in case that the recovery of radioactivity was not satisfactory until Day 8. For the 24-hour visits (if applicable): feces collection to be collected at home within 48 hours prior to admission.

TABLE OF CONTENTS

TABLE OF TABLES

LIST OF ABBREVIATIONS

AME	absorption, metabolism, and excretion
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BMI	body mass index
CA	Competent Authority
CCMO	Centrale Commissie Mensgebonden Onderzoek (Central Committee on Research Involving Human Subjects)
CHMP	Committee for Medicinal Products for Human Use
GMP	Good Manufacturing Practice
CSP	clinical study protocol
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CTD	Clinical Trial Directive
DprE1	decaprenylphosphoryl-β-D-ribose-2'-epimerase
ECG	electrocardiogram
eCRF	electronic case report form
EDS	Early Development Services
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HED	human equivalent dose
HKI	Hans Knöll Institut
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
MEB	Medicine Evaluation Board
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no observed adverse effect level
PCR	polymerase chain reaction
PK	pharmacokinetic(s)
PRA	PRA Health Sciences
SAE	serious adverse event
SAP	statistical analysis plan
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
WMA	World Medical Association
WMO	Wet medisch-wetenschappelijk onderzoek met mensen (medical research involving human subjects act)

Note: Definitions of pharmacokinetic (PK) parameters are provided in Section 3.5.3.

1. INTRODUCTION

1.1 Background

BTZ-043 is a promising new antibiotic for the treatment of tuberculosis. Its mechanism of action is based on a reduction of BTZ-043 to a transient nitroso moiety, which then reacts with an essential cysteine residue in the enzyme decaprenylphosphoryl-β-D-ribose-2'-epimerase (DprE1), forming a semimercaptal as covalent adduct. DprE1 itself is required for the biosynthesis of decaprenylphosphoryl-β-D-arabinose, an essential component for the cell wall assembly of mycobacteria. Formation of the covalent adduct between BTZ-043 and DprE1 results in inhibition of cell wall biosynthesis and loss of viability of *M. tuberculosis*. Due to this mechanism BTZ-043 is highly selective for mycobacteria species (and some other members of Actinomycetales), but does not affect the gut flora.[1]

In 2018 a Phase 1a study entitled “A Randomized, Double blind, Placebo-controlled, Single Ascending Dose Study to Evaluate Safety, Tolerability, and Pharmacokinetics (PK) of Single Doses of BTZ-043 in Healthy Adult Volunteers” (LMU-IMPH-BTZ-043-01) was conducted in humans and currently a Phase 1a/2b study entitled “A Prospective Phase 1b/2a, Active-controlled, Randomized, Open-label Study to Evaluate the Safety, Tolerability, Extended Early Bactericidal Activity and PK of Multiple Oral Doses of BTZ-043 Tablets in Subjects With Newly Diagnosed, Uncomplicated, Smear-positive, Drug-susceptible Pulmonary Tuberculosis” (PanACEA-BTZ-043-02) is ongoing. This human absorption, metabolism, and excretion study is conducted during Phase 2 proof of concept studies and before a Phase 3 study is initiated.

1.1.1 Nonclinical Summary

Safety

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Pharmacokinetics

[redacted]

[redacted]

1.1.2 Clinical Summary

Safety

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted].

[redacted].

[redacted]

1.2 Risk-benefit Assessment

There is no expected clinical benefit for the healthy subjects who will participate in this study. The information obtained in this study can be used for the further clinical development of BTZ-043. As depicted in Table 2, BTZ-043 has been well tolerated in much higher exposures as proposed in this study.

Overall, on the basis of the available nonclinical and clinical data, the risk-benefit profile of BTZ-043 is judged acceptable for the proposed clinical study.

The risk-benefit assessment for the subjects receiving BTZ-043 remains unchanged in relation to the corona virus disease-19 (COVID-19) pandemic as available clinical and nonclinical results do not suggest that administration of BTZ-043 will lead to suppression or modulation of the immune system. In addition, the mode of action does not appear to have a substantial effect on the respiratory or cardiovascular system critically affected by a SARS-CoV-2 infection. As the subjects to be included in this study are in general young to middle aged without major comorbidities, the study population is not considered to be a high-risk population for serious COVID-19 disease. Only persons with a negative SARS-CoV-2 test at admission to the clinical research center will be allowed to participate in the study. In addition, all appropriate measures to prevent SARS-CoV-2 infection during the study will be taken as detailed in Section 3.2.1.

1.3 Study Rationale

This study will determine the disposition and metabolism of BTZ-043 in humans after oral administration. The use of radiolabeled molecules (usually [^{14}C]) is a common method to ascertain information on the elimination routes and metabolic fate of a compound at an early stage of development. This evaluation will provide an estimate of PK parameters, the routes and rates of elimination of radioactivity, and identification of metabolites and metabolic pathways. These data will be compared with similar data obtained from nonclinical studies, both from PK and metabolic points of view. The rationale for the proposed dose can be found in Section 3.4.4.

2. OBJECTIVES

2.1 Primary

- To determine the rates and routes of excretion of [^{14}C]BTZ-043-related radioactivity, including mass balance of total drug-related radioactivity in urine and feces (and vomit, if applicable), following the oral administration of a single 500 mg dose of [^{14}C]BTZ-043 in healthy male volunteers.
- To determine the PK of total radioactivity in whole blood and in plasma.
- To characterize the plasma PK of BTZ-043 and main metabolites by liquid chromatography-mass spectrometry (LC-MS), if applicable.
- To characterize the urine concentrations of BTZ-043 and main metabolites by LC-MS, if applicable.

2.2 Secondary

- To assess the safety and tolerability of a single 500 mg oral dose of BTZ-043 administered to healthy male volunteers.

2.3 Exploratory

- To identify BTZ-043's metabolites in plasma, and to quantify metabolites not measured in the primary objectives by radiometry in order to elucidate key biotransformation pathways and clearance mechanisms of BTZ-043 in humans.
- To identify BTZ-043's metabolites in excreta (urine and feces) and to quantify metabolites not measured in the primary objectives by radiometry in order to elucidate key biotransformation pathways and clearance mechanisms of BTZ-043 in humans.

3. INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

3.1.1 Type of Study

This will be a Phase 1, single-center, open-label, absorption, metabolism, and excretion (AME) study in 4 healthy adult male subjects.

3.1.2 Screening Period

Subjects will report to the medical screening facility/clinical site for the eligibility screening (see Section 3.3 for inclusion and exclusion criteria) within 3 weeks prior to drug administration.

Subjects will sign the study-specific informed consent form (ICF) prior to any study-specific screening procedures being performed. The written informed consent will be obtained for all subjects, regardless of their eligibility for the study; the signed ICFs will be retained and archived at PRA Health Sciences (PRA) and a copy will be provided to the subject.

Eligibility screening will consist of the assessments as presented in the schedule of assessments (Table 1).

3.1.3 Treatment Period

Subjects will be admitted to the clinical research center on Day -1, which is the day prior to Day 1, the day of drug administration.

Subjects will receive a single oral dose of 500 mg BTZ-043 containing 3.7 MBq of [^{14}C]BTZ-043 on Day 1. Subjects will be discharged from the clinical research center on Day 5 (approximately 96 hours after drug administration) upon satisfactory recovery of radioactivity. If the criteria for discharge are not met on Day 5, subjects will be required to remain confined for a maximum of 3 additional days (Days 6 to 8) until the criteria are met (daily check by quick counts or normal counts, as feasible). The in-house period should be prolonged up to the morning of Day 8 if at least 1 of the following applies:

- Total radioactivity in plasma is higher than 5% of radioactivity-C_{max} and/or PK parameters for determination of the total radioactivity-elimination phase cannot be determined reliably.
- The mass balance in urine and feces is not complete by Day 5 (ie, >90%).
- The combined urinary and fecal excretion is not ≤1% of the administered dose per 24 hours for 2 consecutive days based on ^{14}C-radioactivity “quick counts” or normal counts, as feasible.

If the criteria for discharge are still not met on Day 8, subjects may be discharged from the clinical site upon discretion of the Investigator. To ensure that adequate consideration is given to the protection of the subject’s interests, subjects with an incomplete mass balance on Day 8 will be asked to return to the clinical site for one or two 24-hour visits (Day 11-12 and Day 15-16).

On the day of study drug administation (Day 1), an FDA-recommended high-fat (or a standardized breakfast, if applicable, as outlined in Section 3.4.4 and Section 3.4.7) with calculated caloric content will be provided.

Assessments during the treatment period will be performed as presented in the schedule of assessments (Table 1).

3.1.4 Follow-up

The follow-up assessments will be performed 30 days (±2 days) after the [^{14}C]BTZ-043 dose.

Assessments during follow-up will be performed as presented in the schedule of assessments (Table 1).

3.2 Discussion of Study Design

The current design is commonly used for AME studies and in accordance with the current EMA "Guideline on the investigation of drug interactions". [2] Since the present study is a descriptive study aimed at assessing excretion routes of BTZ-043 and evaluating mass balance, a formal statistical sample size calculation was not performed. A sample size of 4 subjects is a minimum, accepted number of subjects for AME studies and is considered sufficient to achieve the study objectives. Early-termination subjects may be replaced if the total number of completers drops below 4, after mutual agreement between the Sponsor and PRA.

3.2.1 COVID-19 Risk Mitigation

This study will be conducted in accordance with guidance from the Central Committee on Research Involving Human Subjects (CCMO [Centrale Commissie Mensgebonden Onderzoek], ie, the Dutch competent authority [CA]) on conducting Phase 1 trials in clinical research centers in The Netherlands during the COVID-19 pandemic.

During the entire study, the clinical research center will implement all recommendations issued by the Dutch government, including specific guidelines related to clinical research executed in clinical research centers with respect to minimizing the risk of disease spreading (eg, social distancing, disinfection, hygiene, and wearing of personal protection equipment by study staff). Details on specific procedures are described in the site specific manual.

In cases where subjects are not able to attend study visits due to an infection with SARS-CoV-2, the Investigator will discuss with the Sponsor potential mitigation approaches (including, but not limited to, extending the visit window, conducting evaluations via video link or phone call, allowing for safety procedures to be conducted at a local facility). The rationale (eg, the specific limitation imposed by the SARS-CoV-2 infection that led to the inability to perform the protocol-specified assessment) and outcome of the discussion will be documented in the electronic case report form (eCRF).

In addition, the following containment measures will be taken during the study:

- Polymerase chain reaction (PCR) testing for SARS-CoV-2 will be performed at the time points indicated in Table 1.
- A subject should not be admitted if there was any contact with a person who tested positive for SARS-CoV-2 or a COVID-19 patient within the last 2 weeks prior to admission to the clinical research center.
- If a subject is tested to be SARS-CoV-2 positive on Day -1, the subject will be excluded from participation with reference to Exclusion Criterion 13, and referred for treatment.
- Physical examinations will be limited.
- If a subject becomes ill and/or is tested to be SARS-CoV-2 positive after the administration of study treatment, study assessments will be stopped (see also Section 3.3.3.1). A safety follow-up can be done if deemed necessary by the Investigator. The subject will be kept in-house in quarantine with appropriate medical intervention until complete elimination of the study compound or will be asked to quarantine at home according to guidelines of the Dutch government.

3.3 Selection of Study Population

3.3.1 Inclusion Criteria

Note: Restrictions that apply to the period after admission are described in Section 3.4.9, except when they concern a statement of willingness.

The following inclusion criteria must be met for a subject to be eligible for inclusion in the study:

1. Sex : male
2. Age : 18 years to 55 years, inclusive, at screening.
3. Body mass index (BMI) : 18.0 to 29.0 kg/m^2, inclusive, at screening.
4. Weight : 55 to 90 kg, inclusive, at screening.
5. Status : healthy subjects.
6. Male subjects, if not surgically sterilized, must agree to use adequate contraception and not donate sperm from admission to the clinical research center until 90 days after the follow-up visit. Adequate contraception for the male subject (and his female partner, if she is of childbearing potential) is defined as using hormonal contraceptives or an intrauterine device combined with at least 1 of the following forms of contraception: a diaphragm, a cervical cap, or a condom. Total abstinence, in accordance with the lifestyle of the subject, is also acceptable.
7. All prescribed medication must have been stopped at least 30 days prior to admission to the clinical research center.
8. All over-the-counter medications, vitamin preparations (especially vitamin C), other food supplements, and herbal medications (eg, St. John's wort) must have been stopped at least 14 days prior to admission to the clinical research center. An exception is made for paracetamol, which is allowed up to 48 hours prior to study drug administration.
9. No vaccination within 14 days prior to study drug administration.

10. Ability and willingness to abstain from alcohol from 48 hours (2 days) prior to screening and admission to the clinical research center.
11. Ability and willingness to abstain from methylxanthine-containing beverages or food (coffee, tea, cola, chocolate, and energy drinks), grapefruit (juice), corn (whole corn kernels and popcorn), cruciferous vegetables, and bitter oranges from 48 hours (2 days) prior to admission to the clinical research center.
12. Good physical and mental health on the basis of medical history, physical examination, clinical laboratory, ECG, and vital signs, as judged by the Investigator.
13. Willing and able to sign the ICF.

3.3.2 Exclusion Criteria

Note: Restrictions that apply to the period after admission are described in Section 3.4.9, except when they concern a statement of willingness.

A subject who meets any of the following exclusion criteria will not be eligible for inclusion in the study:

1. Participation in another study with a radiation burden of >0.1 mSv and ≤1 mSv in the period of 1 year prior to screening; a radiation burden of >1.1 mSv and ≤2 mSv in the period of 2 years prior to screening; a radiation burden of >2.1 mSv and ≤3 mSv in the period of 3 years prior to screening, etc.
2. Exposure to radiation for diagnostic reasons (except dental X-rays and plain X-rays of thorax and bony skeleton [excluding spinal column]), or during work within 1 year prior to drug administration.
3. Irregular defecation pattern (less than once per day on average).
4. Employee of PRA, Nuvisan, or the Sponsor.
5. History of relevant drug and/or food allergies.
6. Using tobacco products within 60 days prior to drug administration.
7. History of alcohol abuse or drug addiction (including soft drugs like cannabis products).
8. Positive drug and alcohol screen (opiates, methadone, cocaine, amphetamines [including ecstasy], cannabinoids, barbiturates, benzodiazepines, gamma-hydroxybutyric acid, tricyclic antidepressants, and alcohol) at screening or admission to the clinical research center.
9. Average intake of more than 24 grams of alcohol per day.
10. Positive screen for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibodies, or HIV 1 and 2 antibodies.
11. Participation in a drug study within 30 days prior to drug administration in the current study. Participation in more than 4 drug studies in the 12 months prior to drug administration in the current study.
12. Donation or loss of more than 450 mL of blood within 60 days prior to drug administration. Donation or loss of more than 1.5 liters of blood in the 10 months prior to drug administration in the current study.
13. Significant and/or acute illness within 5 days prior to drug administration that may impact safety assessments, in the opinion of the Investigator.

14. Unwillingness to consume the Food and Drug Administration (FDA)-recommended high-fat breakfast.
15. Unsuitable veins for infusion or blood sampling.
16. Positive nasopharyngeal PCR test for SARS-CoV-2 on Day -1.

Please note that subjects should refrain from consumption of any foods containing poppy seeds, corn (whole corn kernels and popcorn), cruciferous vegetables, and bitter oranges within 48 hours (2 days) prior to screening and admission to the clinical research center to avoid false positive drug screen results, difficult feces texture, or induction of drug metabolizing enzymes. In addition, they should refrain from strenuous exercise within 96 hours (4 days) prior to screening and admission as this could result in abnormal clinical laboratory values.

3.3.3 Removal of Subjects from Assessment

Participation in the study is strictly voluntary. A subject has the right to withdraw from the study at any time for any reason.

The Investigator has the right to terminate participation of a subject for any of the following reasons: difficulties in obtaining blood samples, violation of the protocol, severe AEs or SAEs, or for any other reason relating to the subject's safety or integrity of the study data.

If a subject is withdrawn from the study, Nuvisan and LMU Klinikum will be informed immediately. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned, or the condition has stabilized with no more change being likely.

After signing informed consent, subjects who drop out or withdraw for any reason without completing all screening evaluations successfully as well as subjects who drop out or withdraw prior to the first dose of study drug will be considered screening failures.

A subject who is withdrawn or voluntarily withdraws from the study for any reason, whether related to the study drug or not, after having received the study drug, will be considered an early-termination subject. If a subject is withdrawn from the study, the early-termination subject will be replaced after mutual agreement between the Sponsor and PRA if the number of evaluable subjects drops below 4 (with a maximum of 2 replacements).

The decision regarding the replacement of subjects will be documented.

PRA will make every effort to ensure that early-termination subjects who have received the study drug complete the early-termination assessments/safety follow-up assessments.

Early-termination subjects may be replaced if the total number of completers drops below 4, after mutual agreement between Nuvisan, LMU Klinikum, and PRA.

3.3.3.1 Stopping Rules for Individual Subjects

In case of a positive SARS-CoV-2 virus PCR test or evidence for COVID-19 during the study, the subject will be withdrawn from the study. The subject will be isolated from other study participants and referred for treatment. The subject will be followed up in quarantine in the clinical research center until complete elimination of the study compound or will be asked to quarantine at home according to guidelines of the Dutch government.

3.4 Treatments

3.4.1 Treatments Administered

Subjects will receive a single oral dose of 500 mg BTZ-043 containing 3.7 MBq of [^{14}C]BTZ-043 on Day 1.

3.4.2 Identity of Investigational Product

Active Medication

Active substance	:	BTZ-043
Activity	:	Inhibition of *M. tuberculosis* DprE1
In development for	:	Tuberculosis
Dose	:	1 x 100 mL (equivalent to 500 mg BTZ-043)
Strength	:	5 mg/mL BTZ-043 containing 0.037 MBq of [^{14}C]BTZ-043
Dosage form	:	Oral suspension
Manufacturer	:	Sourced by pharmacy at PRA

The active pharmaceutical ingredient will be provided by the Promotor.

[^{14}C]BTZ-043 is a drug under development and not approved yet. It will be manufactured and analysed according to Good Manufacturing Practice (GMP) requirements. A GMP certificate of the manufacturer of the [^{14}C]BTZ-043 suspension and an Investigator's Brochure that summarizes preclinical and clinical data with respect to BTZ-043 are in place. A certificate of analysis and a certificate of conformity will be provided by PRA prior to clinical part.

For details concerning drug storage and drug accountability see Appendix 8.1.

3.4.3 Method of Assigning Subjects to Treatment Groups

After obtaining informed consent, subjects will receive a screening number and will be screened according to the inclusion and exclusion criteria. Subjects who have met all eligibility criteria will receive a subject number upon inclusion in the study (Subject Numbers 001-004). They will receive the subject number just prior to dosing. The subject number will ensure identification throughout the study after study drug administration. Replacement subjects will receive the number of the subject to be

replaced, increased by 100 (eg, 101 replacement number for Subject Number 001), and will be administered the same treatment.

After signing informed consent, subjects who drop out or withdraw for any reason without completing all screening evaluations successfully as well as subjects who drop out or withdraw prior to the first dose of study drug will be considered screening failures. Such subjects, and also subjects who are eligible for inclusion in the study but do not receive the study drug, will not receive a subject number, and only applicable data will be entered in the eCRFs.

3.4.4 Selection of Doses and Meals in the Study

The targeted dose of 500 mg is considered a clinically relevant dose for the human AME study (Table 2) since this exposure achieves a maximal bactericidal effect and has shown to be tolerable in the 14-day Phase 1b study. It is proposed that the active pharmaceutical ingredient is taken after a high-fat breakfast (see Section 3.4.7), as absorption is then much improved. As shown in Table 2, the expected exposure is well below the maximum and well tolerated exposure achieved in the Phase 1b study. In the unexpected case that the currently conducted Phase 2a study with 250 mg, 500 mg, and 1000 mg BTZ-043 administered after a high-fat breakfast shows any dose-related safety alert, the study team and the Sponsor will take measures to reduce the exposure. Reduction of exposure will be achieved by adapting the food intake (up to fasting) before the administration of [^{14}C]BTZ-043. This protocol describes the drug administration of 500 mg [^{14}C]BTZ-043 after a high-fat breakfast as default. However, the study team and the Sponsor will decide whether a reduction in food intake is necessary to reduce the exposure to [^{14}C]BTZ-043.

In case it is indeed decided to administer 500 mg [^{14}C]BTZ-043 after a standardized breakfast or in the fasted state rather than after a high-fat breakfast as planned, this decision and the associated rationale will be laid down in a Note to File, to be signed by the Sponsor and the PI, and to be submitted to the IEC for information only.

The dose of ^{14}C (approximately 3.7 MBq) to be administered was chosen to ensure successful profiling and identification of metabolites.

In the present study, the estimated effective radiation burden after a single oral radioactivity dose of 3.7 MBq is approximately 0.16 mSv. [3] For biomedical investigations in small groups of human volunteers, an effective dose of 0.1 to 1.0 mSv is considered acceptable.

3.4.5 Radiation Safety of Clinical and Laboratory Staff

It is expected that the clinical and laboratory staff handling the radioactive material and samples will not be exposed to any relevant radiation and associated health risk. The [^{14}C] isotope emits low energy beta particles, which are not able to penetrate the dose containers or sample container walls. Therefore, there will be no “external” exposure from sample handling. The samples from the participants after

radiolabeled dose administration will contain low levels of radioactivity. The radioactive dose of 3.7 MBq [^{14}C] per subject is below the legal handling limit, above which a special laboratory and special procedures for handling radioactivity would be required. Therefore, the staff can handle the study samples like any other non-radioactive biological material. Common practice of safe laboratory technique and hygiene will be exercised in handling all biological materials from this study.

3.4.6 Timing of Doses in the Study

On Day 1, the study drug will be administered with the subject in the upright position. The study drug will be administered to subjects between 08:00 and 11:00 in the morning. After an overnight fast of at least 10 hours, subjects will receive an FDA-recommended high-fat breakfast (or a standardized breakfast, if applicable as outlined in Section 3.4.4) that will have to be finished within 20 minutes. The entire breakfast must be consumed by the subjects. The actual consumed food will be documented.

Dosing will occur at 30 minutes after the start of the high-fat breakfast (or the standardized breakfast, if applicable, or dosing will occur in a fasted state, if applicable as outlined in Section 3.4.4).

The study drug will be given as an oral suspension of 100 mL, which will be provided in an amber glass bottle from which the subjects can drink. Thereafter the bottle will be rinsed twice with 70 mL of water, and the subjects will be required to drink the bottle contents after rinsing. Thereafter, the subjects are required to drink additional non-carbonated water until they have ingested a total volume of 380 mL.

Following drug administration, subjects will fast for a period of 4 hours until lunch. During fasting, no fluids are allowed except water; however, water is not allowed from 1 hour predose until 1 hour postdose (apart from the water taken with the dose).

Subjects will not lie down for 4 hours after drug administration, except when required for assessments that need to be performed.

3.4.7 Meals During the Study

A fasting period of at least 4 hours is required before obtaining clinical laboratory blood samples at the time points indicated in the schedule of assessments (Table 1).

An FDA-recommended high-fat (or a standardized breakfast, if applicable, as outlined in Section 3.4.4) with calculated caloric content will be provided on Day 1.

The FDA-recommended high-fat breakfast of 918 kcal consists of:

- 2 fried eggs (in 15 g butter/margarine) (approximately 100 g)
- 1 portion of bacon (40 g) (or brie 60+ for vegetarians)
- 1 portion of fried potatoes (115 g)
- 2 slices of (toasted) (wheat) bread with 15 g margarine
- 1 glass of whole milk (240 mL)

The total of 918 kcal (vegetarian version 915 kcal) can be broken down as follows:

- 39 g protein = 156 kcal
- 59 g fat = 527 kcal
- 59 g carbohydrates = 235 kcal

The standardized breakfast of 572-755 kcal (depending on choices made) consists of:

- 3 slices of whole-wheat bread
- 1 Dutch rusk
- 20 g halvarine/margarine
- 1 portion of cheese/old cheese/cheese spread
- 1 portion of ham/chicken breast/smoked meat/spreadable liver sausage/baloney/filet American/salami (or cheese, cheese spread, or peanut butter for vegetarians)
- 2 portions of jam or other sweet spread
- 2 mugs of decaffeinated coffee/tea (cream and sugar optional, max 2 of both)

The total of 572-755 kcal (vegetarian version 589-805 kcal) can be broken down as follows:

- 18-19 g protein = 71-75 kcal
- 26-32 g fat = 233-292 kcal
- 67-97 g carbohydrates = 268-388 kcal

Other meals and snacks (such as decaffeinated coffee, herbal tea, fruit, and biscuits) will be provided according to PRA standard operating procedures (SOPs), taking into account the restrictions as described in Section 3.4.9 and what has been described in Section 3.4.6.

3.4.8 Blinding

This is an open-label study.

3.4.9 Concomitant Medication and Other Restrictions During the Study

Note: Restrictions that apply to the period before admission are described in Section 3.3.1 and Section 3.3.2.

The use of all prescribed medication is not allowed from admission to the clinical research center until follow-up. The use of all over-the-counter medications, vaccines, vitamin preparations (especially vitamin C), other food supplements, and herbal medications (eg, St. John's wort) is not allowed from admission to the clinical research center until follow-up. An exception is made for paracetamol: from admission onwards, the Investigator may permit a limited amount of paracetamol for the treatment of headache or any other pain. Other medication to treat AEs may only be prescribed if deemed necessary by the Investigator. If medication is used, the name of the drug, the dose, and dosage regimen will be recorded in the eCRF.

The use of methylxanthine-containing beverages or food (coffee, tea, cola, chocolate, and energy drinks), grapefruit (juice), kiwi, and tobacco products is not allowed during the stay in the clinical research center.

The use of alcohol is not allowed during the stay in the clinical research center and within 48 hours (2 days) prior to the 24-hour return visits (Days 11-12 and 15-16, as needed) and prior to follow-up.

Strenuous exercise is not allowed within 96 hours (4 days) prior to admission and follow-up. Strenuous exercise is also not allowed during the stay in the clinical research center.

Subjects should not consume any foods containing poppy seeds, corn (whole corn kernels and popcorn), cruciferous vegetables, and bitter oranges within 48 hours (2 days) prior to admission to the clinical research center as this could cause a false positive drug screen result.

Male subjects, if not surgically sterilized, are required to use adequate contraception (see Inclusion Criterion 6) and not donate sperm from admission to the clinical research center until 90 days after the follow-up visit.

Subjects must not donate blood during the study until the follow-up visit (other than the blood sampling planned for this study).

3.4.10 Treatment Compliance

Study drug will be administered in the clinical research center. To ensure treatment compliance, administration of the study drug will be supervised by the Investigator or an authorized designee. Compliance will be further confirmed by bioanalytical assessment of BTZ-043 and total radioactivity in plasma and urine samples (see Section 3.5.4).

The exact times of study drug administration and the number of units administered will be recorded in the eCRF.

3.5 Pharmacokinetic, Safety, and Exploratory Measurements and Variables

3.5.1 Pharmacokinetic and Safety Measurements Assessed and Schedule of Assessments

A schedule of assessments is presented in Table 1.

3.5.1.1 Pharmacokinetic Measurements

3.5.1.1.1 Blood Sampling

At the time points defined in the schedule of assessments (Table 1), blood samples will be taken for the analysis of BTZ-043 and metabolites in plasma samples, total radioactivity in whole blood and plasma samples, and metabolite profiling and identification in plasma samples.

The blood samples will be taken via an indwelling iv catheter or by direct venipuncture. The exact times of blood sampling will be recorded in the eCRF.

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA and Nuvisan.

3.5.1.1.2 Urine Collection

During the intervals defined in the schedule of assessments, urine will be collected for the analysis of BTZ-043 and metabolites, for total radioactivity, for quick counts or normal counts of total radioactivity (as feasible), and for metabolite profiling and identification. The subjects will be instructed to empty their bladders completely before drug administration and at the end of each collection interval. A baseline urine sample will be collected within 12 hours prior to drug administration. The exact times of urine collection and the urine weight of the entire interval will be recorded in the eCRF.

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA and Nuvisan.

3.5.1.1.3 Feces Collection

During the intervals defined in the schedule of assessments, all fecal excretions will be collected for the analysis of total radioactivity, for quick or normal counts of total radioactivity (as feasible), and for metabolite profiling and identification. A blank fecal sample will be collected within 48 hours prior to study drug administration. The exact times of feces collection and the fecal weight will be recorded in the eCRF.

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA and Nuvisan.

3.5.1.1.4 Vomit Collection

If a subject vomits within the time period from drug administration up to 8 hours after the administration of the study drug, the vomit should, if possible, be collected. Any collected vomit will be included in the total radioactivity measurements and in the determination of the mass balance. The exact times of vomit production will be recorded in the eCRF.

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA and Nuvisan.

3.5.1.2 Safety and Tolerability Measurements

Safety and tolerability assessments will consist of AEs, clinical laboratory, vital signs, 12-lead ECG, and physical examination. Assessments will be performed in accordance with the schedule of assessments.

3.5.1.2.1 Adverse Events

AEs will be recorded from signing the ICF until completion of the follow-up visit. Any clinically significant observations, as determined by the Investigator, in results of clinical laboratory, 12-lead ECGs, vital signs, or physical examinations will be recorded as AEs.

A TEAE is defined as any event not present prior to administration of the study drug or any event already present that worsens in either severity or frequency following exposure to the study drug.

An AE that occurs prior to administration of the study drug will be considered a pretreatment AE.

At several time points before and after drug administration, subjects will be asked nonleading questions to determine the occurrence of AEs. Subjects will be asked in general terms about any AEs at regular intervals during the study. In addition, all AEs reported spontaneously during the course of the study will be recorded. All answers will be interpreted by the Investigator using the Medical Dictionary for Regulatory Activities (MedDRA; most recent version) for AEs and will be recorded in the eCRF as reported terms.

The severity of the AEs will be rated as mild, moderate, or severe; the relationship between the AEs and the study drug will be indicated as related or not related. Details on rating the severity of AEs and relationship to study treatment are given in Appendix 8.2.

Pregnancy of female partners of male subjects will be monitored along with follow-up, if warranted (see Appendix 8.3).

3.5.1.2.2 Clinical Laboratory

Blood and urine samples for clinical laboratory assessments will be collected according to PRA SOPs.
The following parameters will be measured:

- Clinical chemistry (serum quantitatively):
  total bilirubin, alkaline phosphatase, gamma glutamyl transferase, AST, ALT, lactate dehydrogenase, creatinine, urea, total protein, glucose, inorganic phosphate, sodium, potassium, calcium, and chloride.
- Hematology (blood quantitatively):
  leukocytes, erythrocytes, hemoglobin, hematocrit, thrombocytes, absolute partial automated differentiation (lymphocytes, monocytes, eosinophils, basophils, and neutrophils), mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.
- Urinalysis (urine qualitatively):
  hemoglobin, urobilinogen, ketones, glucose, protein.
- Serology:
  HBsAg, HCV antibodies, and HIV 1 and 2 antibodies.

- Drug and alcohol screen:
  opiates, methadone, cocaine, gamma hydroxybutyric acid, amphetamines (including ecstasy), cannabinoids, barbiturates, benzodiazepines, tricyclic antidepressants, nicotine metabolites, and alcohol.

Nasal and throat mucosal cell samples will be collected according to PRA work instructions detailed in the site specific manual. The samples will be tested for SARS-CoV-2 virus using PCR tests. The nonclinical project manager will be informed immediately upon availability of Day 11 results, as this will qualify the feces samples for metabolite profiling with regard to potential COVID-19 infectivity.

In case of unexplained or unexpected clinical laboratory test values, the tests will be repeated as soon as possible and followed up until the results have returned to the normal range and/or an adequate explanation for the abnormality is found. The clinical laboratory will clearly mark all laboratory test values that are outside the normal range, and the Investigator will indicate which of these deviations are clinically significant. Clinically significant laboratory result deviations will be recorded as AEs, and the relationship to the treatment will be indicated (see also Appendix 8.2).

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA.

3.5.1.2.3 Vital Signs

Systolic and diastolic blood pressure and pulse will be recorded after the subject has been resting for at least 5 minutes in the supine position. These assessments will be made using an automated device whenever possible. Body temperature and respiratory rate will also be measured.

3.5.1.2.4 Electrocardiogram

A standard 12-lead ECG will be recorded after the subject has been resting for at least 5 minutes in the supine position. The ECG will be recorded using an ECG machine equipped with computer-based interval measurements. The following ECG parameters will be recorded: heart rate, PR-interval, QRS-duration, QT-interval, QTc-interval (Fridericia's), and the interpretation of the ECG profile by the Investigator.

3.5.1.2.5 Physical Examination

Physical examination will be performed according to PRA SOPs. In addition, body weight and height will be measured according to PRA SOPs.

3.5.1.3 Total of Blood Volume

Table 3 presents the number and volume of blood samples and the total volume of blood that will be collected throughout the study per subject.

If deemed necessary by the Investigator or the Sponsor, the number and/or volume of blood samples per assessment may be increased, as long as the total volume of blood

drawn for a subject does not surpass 500 mL (except when extra blood samples need to be taken for safety reasons).

Table 3 Number and Volume of Blood Samples and Total Blood Volume Collected per Subject

Assessment	Maximum # Samples	Volume of Blood per Sample (mL)	Total Volume of Blood (mL)
Pharmacokinetics - BTZ-043 and metabolites in Plasma - Total Radioactivity in Plasma - Total Radioactivity in Whole Blood - Metabolite Profiling in Plasma	14 21 21 ~~21~~19	~~4~~2 ~~2~~4 1 ~~12~~15	~~56~~28 ~~42~~84 21 ~~252~~285
Clinical Chemistry	4	3.5	14
Hematology	4	3	12
Serology	1	5	5
Total Volume of Blood Drawn			~~402~~449

3.5.2 Appropriateness of Measurements

The assessments that will be made in this study are standard and generally recognized as reliable, accurate, and relevant.

3.5.2.1 Timing of Assessments

For PK, predose samples will be obtained between waking up and dosing. Postdose samples up to 20 minutes postdose will be obtained with a time window of ±1 minute. Thereafter, postdose samples will be obtained with time margins of ±5% of the time that has passed since dosing. The ±5% time window also applies to the start and end times of urine collection intervals and to the total duration of each collection interval.

For safety assessments, predose assessments will be performed between waking up and dosing. For safety assessments up to 2.5 hours postdose, a time window of ±15 minutes is allowed. Thereafter, serial postdose assessments (eg, multiple assessments within any given day) will be performed with time margins of ±10% of the time that has passed since dosing.

When assessments are planned for the same scheme time, the order of the assessments should be arranged in such a way that PK blood sampling will be done after the ECG and vital signs recordings have been conducted, with PK blood sampling exactly on time.

3.5.3 Pharmacokinetic, Safety, and Exploratory Variables

3.5.3.1 Pharmacokinetic Variables

PK variables will be the plasma and urine concentrations of BTZ-043 and main metabolites, the total radioactivity concentrations in plasma, whole blood, urine, and feces (and in vomit, if produced) and their PK parameters. The PK parameters to be

determined or calculated using noncompartmental analysis include, but are not limited to, the parameters as given in Table 4. A complete list of PK parameters will be provided in the PK statistical analysis plan (SAP).

Table 4 Pharmacokinetic Parameters

Parameter	Description	Parent	Metabolites	TRA
C_{max}	Maximum observed plasma concentration	X	X	X
t_{max}	Time to attain maximum observed plasma concentration	X	X	X
AUC_{0-t}	Area under the plasma concentration-time curve up to time t, where t is the last point with concentrations above the LLOQ	X	X	X
AUC_{inf}	Area under the plasma concentration-time curve from time 0 to infinity calculated as: $AUC_{0-inf}=AUC_{0-t}+C_{last}/k_{el}$, where C_{last} is the last measurable plasma concentration	X	X	X
K_{el}	Terminal elimination rate constant	X	X	X
$t_{1/2}$	Terminal elimination half-life, calculated as $0.693/k_{el}$	X	X	X
CL/F	Apparent oral clearance, calculated as dose/AUC_{0-inf}	X		
V_z/F	Apparent volume of distribution at terminal phase	X		
CL_R	Renal clearance	X		
Ae_{urine}	Cumulative amount of drug excreted in urine	X	X	X
Ae_{feces}	Cumulative amount of drug excreted in feces			X
Ae_{vomit}	Cumulative amount of drug excreted in vomit, if produced			X
Ae_{total}	Total amount of drug excreted, calculated as $Ae_{total}=Ae_{urine}+Ae_{feces}(+Ae_{vomit})$			X
fe_{urine}	Fraction of the dose administered excreted in urine (%)	X	X	X
fe_{feces}	Fraction of the dose administered excreted in feces (%)			X
fe_{vomit}	Fraction of the dose administered excreted in vomit (%)			X
fe_{total}	Fraction of the dose administered excreted in urine, feces, and vomit (if produced) (%)			X

TRA=total radioactivity; LLOQ=lower limit of quantification

3.5.3.2 Safety Variables

The safety variables to be measured include, but are not limited to, the variables as given below. A complete list of safety variables will be provided in the SAP.

- AEs
- Clinical laboratory
- Vital signs
- 12-lead ECG
- Physical examination

3.5.3.3 Exploratory Variables

Metabolite profiling and metabolite identification will be conducted under a separate protocol/bioanalytical study plan, by Nuvisan Grafing, and the results will be reported separately. ~~The metabolite profiling/identification report will be included in the clinical study report (CSR) as an appendix, and results from the metabolite profiling/identification report will be briefly summarized in the CSR.~~

3.5.4 Drug Concentration Measurements

The analysis of BTZ-043 and main metabolites in plasma and urine samples will be performed at the Bioanalytical Department of Nuvisan Grafing using validated/qualified LC-MS. The analysis of whole blood, plasma, urine, and feces (and vomit, if produced) for total radioactivity will be conducted at the Bioanalytical Laboratory of PRA using a validated liquid scintillation counting method. The bioanalytical reports for the determinations will be included as appendices in the CSR.

The analysis of quick counts will be conducted at the Bioanalytical Laboratory of PRA using a validated method.

Metabolite profiling in plasma, urine, and feces samples by radiometry will be conducted at the PK Sciences & Biotransformation Department of Nuvisan Grafing. ~~The bioanalytical report for the metabolite profiling and identification will be included as appendix in the CSR.~~

3.5.5 Retention of Blood, Plasma, Urine, and Feces Samples

Blood and urine samples remaining after clinical laboratory assessments have been performed will not be stored for future use and will be destroyed after analysis as per laboratory procedure.

Blood, plasma, urine, and feces (and vomit, if applicable) samples remaining after total radioactivity measurements and after sample aliquotation for bioanalytical and metabolite identification have been performed, will be stored at the site of the Promotor and destroyed no later than 10 years after the date of the final CSR.

3.6 Statistical Procedures and Determination of Sample Size

3.6.1 Analysis Sets

3.6.1.1 Safety Set

All subjects who have received the single oral dose of ^{14}C-BTZ-043.

3.6.1.2 Pharmacokinetic Set

All subjects who have received the single oral dose of ^{14}C-BTZ-043 and provided sufficient bioanalytical assessment results to calculate reliable estimates of the PK parameters.

3.6.2 Statistical and Analytical Plan for Pharmacokinetic, Safety, and Exploratory Evaluation

A safety SAP will be generated by the Biostatistics Department of PRA and a PK SAP will be generated by Nuvisan; the SAPs will be finalized prior to database lock. Full details of the analysis to be performed will be included in the SAPs.

Any deviation from the SAPs will be reported in the section “Changes in Planned Analysis” in the CSR.

3.6.2.1 Pharmacokinetic Evaluation

The PK parameters and their statistical evaluation of BTZ-043 and *main* metabolites will be reported as ~~an integrated PK report, which will be included as an appendix and briefly summarized in the CSR.~~ *part of the bioanalytical report (included as appendix in the CSR). The PK parameters and their statistical evaluation of total radioactivity will be part of the mass balance report (included as an appendix in the CSR). A separate integrated PK report will incorporate the BTZ-043 and main metabolites' PK, the total radioactivity PK, and the metabolite profiling PK data for an integrated evaluation.*

All data will be summarized using descriptive statistics and will be listed and summarized in tabular and/or graphical form.

3.6.2.2 Evaluation of Safety and Tolerability

Safety and tolerability will be assessed through AEs, clinical laboratory, vital signs, ECGs, physical examination findings, and any other parameter that is relevant for safety assessment.

3.6.2.2.1 Adverse Events

A listing of all individual AEs will be provided. Summary tables of TEAEs will be presented by system organ class based on the MedDRA terminology list (preferred terms): 1 containing the number of TEAEs (frequency of occurrence, number of subjects experiencing the event) by treatment and 1 containing the number of drug-related TEAEs (frequency of occurrence, number of subjects experiencing the event) per treatment. Additional tables of total counts by treatment and relationship and by treatment and severity will be given.

3.6.2.2.2 Clinical Laboratory

Clinical laboratory data will be listed accompanied by an indication if the parameter is outside the reference range. A summary of all data outside the reference range of the clinical laboratory will be provided. Clinical laboratory data will be presented descriptively, where applicable.

3.6.2.2.3 Vital Signs and Electrocardiograms

Vital signs and ECG parameters will be listed and they will be presented descriptively, where applicable.

3.6.2.2.4 Physical Examination

Changes from baseline for physical examination will be described and listed.

3.6.2.3 Evaluation of Exploratory Variables

The PK parameters and their statistical evaluation of metabolites *not measured for the primary objectives* will be reported in ~~the~~ *a separate* integrated PK report.~~, which will be included as an appendix and briefly summarized in the CSR.~~

All data will be summarized using descriptive statistics and will be listed and summarized in tabular and/or graphical form.

The metabolite profiling and identification results will be reported separately (see Section 3.5.4).

3.6.3 Determination of Sample Size

Since the present study is a descriptive study aimed at assessing excretion routes of BTZ-043 by evaluating mass balance, a formal statistical sample size calculation was not performed. A sample size of 4 subjects is a minimum accepted number of subjects for AME studies and is considered sufficient to achieve the study objectives. Early-termination subjects may be replaced if the total number of completers drops below 4, after mutual agreement between the Sponsor and PRA.

3.7 Data Quality Assurance

The study may be audited by the Quality Assurance Department at PRA to assess adherence to the clinical study protocol (CSP) and Quality System. During the conduct of the study, process-related audits may be performed. An audit certificate will be provided in the appendices of the final CSR outlining any audits and other related activities performed.

The clinical research site will be monitored by the study monitor to ensure correct performance of the study procedures and assure that the study is conducted according to the relevant regulatory requirements. The eCRF entries will be verified with the source documentation, if applicable (in some cases data are captured directly on the eCRF, therefore source data verification is not necessary).

Regulatory authorities, the Independent Ethics Committee (IEC), and/or the Sponsor's clinical quality assurance group may request access to all source documents, eCRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

Quality control principles will be applied throughout the performance of this study. Review procedures will be followed at PRA for all documents that are generated in relation with the study.

An explanation will be given for all missing, unused, and spurious data in the relevant sections of the CSR.

4. ETHICS

4.1 Independent Ethics Committee

The submission package including the CSP and the ICFs will be submitted for review and approval by the IEC of the foundation "Beoordeling Ethiek Biomedisch Onderzoek" (English translation: "Evaluation of Ethics in Biomedical Research") (Dr. Nassaulaan 10, 9401 HK Assen, The Netherlands) prior to the eligibility screening. The composition of the IEC is in accordance with the recommendations of the World Health Organization, the International Council for Harmonisation (ICH) E6(R2) Guideline for Good Clinical Practice (GCP) (European Medicines Agency [EMA]/Committee for Medicinal Products for Human Use [CHMP]/ICH/135/1995)[4], and the EU Clinical Trial Directive (CTD) (Directive 2001/20/EC)[5] (see below). The submission package will also be shared with the CA in the Netherlands for a statement of no objection.

PRA will keep the IEC informed about the progress of the study. All changes in research activities and all unanticipated problems involving risks to human subjects will be immediately reported to the IEC. In accordance with Section 10, Subsection 1, of the Dutch law on Medical Research in Human Subjects (WMO, revised Dec 2015)[6], PRA will inform the subjects and the IEC if anything occurs on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal, or if further recruitment of subjects in the study has been put on hold for that reason, whichever occurs first. The study may be suspended pending further review by the IEC, except insofar as suspension would jeopardize the subjects' health. PRA will take care that all subjects are kept informed.

No changes will be made to the study without IEC approval, except when required to eliminate apparent immediate hazards to human subjects.

Notification of the end of the study will be sent by PRA to the CA in The Netherlands and to the IEC within 90 days after completion of follow-up for the last subject. In case a study is temporarily halted, PRA will notify the IEC immediately, including the reason for this. In case a study is ended prematurely, PRA will notify the IEC and the CA in The Netherlands within 15 days, including the reasons for the premature termination. A summary of the results of the study will be sent by PRA to the CA and the IEC within 1 year after the end of the study.

The written documentation of approval of the CSP and ICFs will be provided to the Sponsor before the start of the study. Any communication with the IEC and/or CA before, during, and/or after the study will be provided to the Sponsor.

4.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origin in the World Medical Association (WMA) Declaration of Helsinki, adopted by the 18th WMA General Assembly, Helsinki, Finland, Jun 1964, and subsequent amendments.[7]

This study is also designed to comply with ICH E6(R2) Guideline for GCP (EMA/CHMP/ICH/135/1995)[4], and the EU CTD Directive 2001/20/EC[5], as incorporated into Dutch Law.[6]

Guidelines adopted by the ICH and other relevant international guidelines, recommendations, and requirements will be taken into account as comprehensively as possible, as long as they do not violate local law.

Whenever the term "Investigator" is noted in the CSP text, it may refer to either the Investigator at the site or an appropriately qualified, trained, and delegated individual of the investigational site.

4.3 Subject Information and Consent

All subjects will be informed verbally and in writing regarding the objectives, procedures, and risks of study participation. The subjects will be given the time to ask questions. The subjects will have to sign the Dutch or English version of the ICF before any study-related procedures are started. The signed ICFs will be retained and archived at PRA and a copy will be provided to the subject. The ICF contains information about the objectives of the study, the procedures followed during the study, and the risks and restrictions of the study, with special reference to possible side effects of the study drug and potential interactions. In addition, insurance coverage provided during the study is explained. The elements addressed in the ICF are according to the ICH E6(R2) Guideline for GCP (EMA/CHMP/ICH/135/1995).[4]

4.4 Privacy

All personal details will be treated as confidential by the Investigator and staff at PRA, the Sponsor, and any sub-contractors involved, and handling of personal data will be in compliance with the EU General Data Protection Regulation.[8]

5. STUDY ADMINISTRATIVE STRUCTURE

5.1 Distribution of Activities

5.1.1 Preparation of Study Drug

The study drug will be prepared at the PRA Pharmacy.

5.1.2 Laboratory Assessments

The analysis of BTZ-043 and main metabolites in plasma and urine samples will be performed at the Bioanalytical Department of Nuvisan Grafing (Grafing, Germany) by using the Nuvisan study protocol G-A-LAB-21-004.

The analysis of total radioactivity in whole blood, plasma, urine, and feces (and vomit, if produced) will be performed at the Bioanalytical Laboratory of PRA (Assen, The Netherlands).

The analysis of clinical laboratory samples will be performed at the PRA Clinical Laboratory.

Metabolite profiling and identification will be performed at the PK Sciences and Biotransformation Department of Nuvisan Pharma Services GmbH (Grafing, Germany) by using the Nuvisan study protocol G-A-MET-21-002.

5.1.3 Electronic Case Report Form Design

The eCRF design will be performed with the computer program Oracle Clinical (Oracle, Redwood Shores, Redwood City, CA, US) by the Database Programming Department of PRA.

5.1.4 Data Management

Data management will be performed with the computer programs Oracle Clinical (Oracle, Redwood Shores, Redwood City, CA, US), SAS® (SAS Institute Inc, Cary, NC, US), and EXACT (Kinship EXACT™, Kinship Technologies, a technology subsidiary of PRA) by the Data Management Department of PRA.

5.1.5 Statistics

A safety SAP will be provided by the Biostatistics Department of PRA. The safety analysis will be conducted by the Biostatistics Department of PRA. Statistical analysis will be performed with the computer program SAS® (SAS Institute Inc, Cary, NC, US). A PK SAP will be provided by Nuvisan Grafing. Statistical evaluation of PK parameters will be conducted by Nuvisan Grafing. PK parameters will be calculated using Phoenix WinNonlin (Pharsight, Mountain View, CA, US). All individual results will be provided to the Sponsor after completion of the study.

5.1.6 Clinical Study Report Writing

The CSR, structured in accordance with the guideline "Structure and Content of Clinical Study Reports - ICH E3"[9], will be written by PRA and a draft version prepared for

approval by the Sponsor. The final version will be provided as paper version to the Sponsor.

5.2 Documentation

5.2.1 Archiving

All documents concerning the study will be kept on file in the Central Archives of PRA for at least 25 years after conduct of the study. The Sponsor will receive the completed eCRFs as PDF file.

5.2.2 Recording of Data in Source Documents and Electronic Case Report Forms

Wherever possible, all data will be entered directly into the eCRFs. Source documents will be used in some cases.

A data management plan will be written by the Data Management Department of PRA, which will be finalized prior to performing any data validation. An appendix to the data management plan (origin of source data list for data entry) will identify any data to be recorded directly in the eCRF (ie, no prior written or electronic record of data) and which data should be considered source data.

6. CONFIDENTIALITY AND PUBLICATION POLICY

All information generated in this study is considered highly confidential and must not be disclosed to any person or entity not directly involved with the study unless prior written consent is gained from the Sponsor and Promotor. However, authorized regulatory officials, the Sponsor and its authorized representatives are allowed full access to the records.

All study subjects must be informed that their personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject, who will be required to give consent for their data to be used as described in the ICF. The subjects must be informed that their medical records may be examined by auditors or other authorized personnel appointed by the Sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

Identification of subjects and eCRFs shall be by unique subject numbers only.

All personal details will be treated as confidential by the Investigator and staff at PRA.

All relevant aspects regarding publication will be part of the contract (or similar document) between the Sponsor and PRA.

7. REFERENCES

1. Investigator's Brochure BTZ-043. Edition 04. 10 Nov 2020.
2. CPMP/EWP/560/95/Rev. 1 Corr. 2** Committee for Human Medicinal Products (CHMP). Guideline on the investigation of drug interactions. 21 Jun 2012.
3. Radiation Burder Calculation Report EDS-NL. Dosimetry BTZ-043. 27 Nov 2018.
4. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E6(R2): Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice. Adopted by the European Medicines Agency (EMA), Committee for Human Medicinal Products, Document Reference EMA/CHMP/ICH/135/1995), 14 Jun 2017.
5. Directive 2001/20/EC of the European Parliament and of the Council of 4 Apr 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.
6. Medical Research Involving Human Subjects Act (WMO, Wet Medisch-Wetenschappelijk Onderzoek met Mensen), revision Dec 2015.
7. World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (18th WMA General Assembly 1964), revised at 64th WMA General Assembly, Fortaleza, Brazil, Oct 2013.
8. The General Data Protection Regulation (GDPR). Regulation (EU) 2016/679 of the European Parliament and the Council of the European Union, 27 Apr 2016, applicable as of 25 May 2018.
9. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E3: Structure and Content of Clinical Study Reports. Note for Guidance on Structure and Content of Clinical Study Reports, Adopted by the Committee for Human Medicinal Products, European Medicines Agency (EMA), Document Reference CPMP/ICH/137/95, Jul 1996.
10. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. Note for Guidance on Clinical Safety Data Management, Adopted by the Committee for Human Medicinal Products, European Medicines Agency (EMA), Document Reference CPMP/ICH/377/95, Jun 1995.

8. APPENDICES

8.1 Drug Accountability

Upon receipt of the study drug, it will be inspected and counted by the responsible pharmacist. If necessary, all study drug will be repacked per dosing occasion and labeled according to PRA SOPs.

The study drug will be kept in the PRA Pharmacy or in a locked and secured storage facility accessible to the pharmacist and the pharmacy assistant only. Temperature is monitored 24/7 with an automatic environmental monitoring system. In case of an alarm, the pharmacist or pharmacist on duty is informed immediately by email and phone.

The responsible pharmacist will keep an inventory. This will include a description of the formulation and the quantity of study drug received for the study and a record of what is dispensed, to whom, and when.

On termination of the study, the responsible pharmacist will conduct a final inventory of the study drug supply and will record the results of this inventory in the Drug Accountability Form. Unused study drug will be returned to the Promotor upon notice is given to Promotor and Sponsor or will be locally destroyed according to PRA standard procedures. Final accountability needs to be verified by monitor before return and/or destruction of medication.

8.2 Adverse Events and Serious Adverse Events Evaluation and Reporting

8.2.1 Adverse Events

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. AE definitions will be followed as stated in the "Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting" (ICH topic E2A).[10]

All AEs reported by the subjects or apparent from their physical appearance during the clinical study will be reported on the AE eCRF page.

The severity of AEs will be graded using the most current version of the MedDRA:

- **Mild**: An AE that is easily tolerated by the subject, causes minimal discomfort, does not interfere with everyday activities, and does not require intervention.
- **Moderate**: An AE that is sufficiently discomforting to interfere with normal everyday activities; intervention may be needed.
- **Severe**: An AE that prevents normal everyday activities; treatment or other intervention usually needed.

If an AE has multiple aspects, the aspect with the highest severity will be graded.

It is emphasized that the term severe is a measure of severity; thus, a severe AE is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

In addition, clinically significant changes in physical examination findings and abnormal objective test findings (eg, laboratory, x-ray, ECG) should also be recorded as AEs. Test findings and physical examination findings can result in AEs if they:

- Are associated with accompanying symptoms, and/or
- Require additional diagnostic testing or medical/surgical intervention, and/or
- Lead to a change in study dosing or discontinuation from the study; result in the addition of significant additional concomitant drug treatment or other therapy, and/or
- Lead to any of the outcomes included in the definition of an SAE, and/or
- Are considered to be an AE by the Investigator or Sponsor.

Reporting as an AE should not be triggered by:

- Merely repeating an abnormal test, or
- Any abnormal test result that is determined to be an error.

The relationship of any AE to the study drug will be assessed and graded as related or not related.

Related:

- The AE follows a reasonable temporal sequence to study drug administration, and cannot be reasonably explained by the subject's clinical state or other factors (eg, disease under study, concurrent diseases, or concomitant medications).
- The AE follows a reasonable temporal sequence to study drug administration, and is a known reaction to the drug under study or a related chemical group, or is predicted by known pharmacology.

Not Related:

The AE does not follow a reasonable sequence from study drug administration, or can be reasonably explained by the subject's clinical state or other factors (eg, disease under study, concurrent diseases, and concomitant medications).

8.2.2 Serious Adverse Events

An SAE is any untoward medical occurrence that, on the basis of medical and scientific judgment:

- Results in death, or
- Is life-threatening (this refers to an event in which the patient 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), or
- Requires inpatient hospitalization for a medical reason or prolongation of existing hospitalization (this refers to hospital admission required for treatment of the AE). (Note: this does not include confinement in, for example, a respite unit; a skilled

nursing unit; rehabilitation facility; the clinical research center; or confinement due to planned or unplanned reason unrelated to study), or

- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Medical and scientific judgment should be exercised in deciding whether expedited 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 patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization.

SAEs will be collected from admission until the final study visit. SAEs that are related to the investigational drug and continue beyond the normal collection period (ie, are ongoing at the time a subject exits the study) will be followed until resolution or until stabilized with sequelae. SAEs that begin after the subject's participation in the study is complete, but that the Investigator considers to be related to study drug, may be reported at any time.

The Investigator or clinical site personnel must notify the Sponsor's Medical Monitor and PRA Drug Safety Center of all SAEs, regardless of relationship to the investigational drug, within 24 hours of clinical site personnel becoming aware of the event. The Investigator will provide the initial notification by sending a completed "SAE Notification Form," which must include the Investigator's assessment of the relationship of the event to investigational drug and must be signed by the Investigator.

In addition, notification is sent by PRA to the IEC and the subject's General Practitioner.

Follow-up information, or new information regarding an ongoing SAE, must be provided promptly to the Sponsor's Medical Monitor and PRA Drug Safety Center.
All SAE reports should be sent to the contacts provided on Page 4: SAE Contact Information.

8.2.3 Suspected Unexpected Serious Adverse Reactions

An SAE that is also an unexpected adverse drug reaction is called a suspected unexpected serious adverse reaction (SUSAR). Unexpected adverse reactions are adverse reactions of which the nature or severity is not consistent with the applicable product information (eg, investigator's brochure for an unapproved investigational medicinal product or the Summary of Product Characteristics for an authorized medicinal product).

The Sponsor or its representative (eg, PRA if agreed to before start of the study) will promptly report (expedited reporting) the following SUSARs to the IEC:

- SUSARs that have arisen in the current clinical study that was assessed by the IEC

- SUSARs that have arisen in other clinical studies of the same Sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the current clinical study that was assessed by the IEC

The Sponsor will promptly report (expedited reporting) all SUSARs to the CA and the Medicine Evaluation Board (MEB) of the country where this study is conducted and to the CAs in other Member States, as applicable.

SUSARs that have already been reported to the EMA Eudravigilance database do not have to be reported again to the CA and the MEB because they have direct access to the Eudravigilance database.

Expedited reporting will occur no later than 15 calendar days after the Sponsor or its representative has first knowledge of the adverse reactions. For fatal or life-threatening cases, the term will be maximally 7 calendar days for a preliminary report with another 8 days for completion of the report.

8.2.4 Follow-up of Adverse Events

Follow-up of AEs will continue until resolution, stabilization, or death. In case of ongoing AEs at database closure, the data obtained at database closure will be used in the statistical analysis. The follow-up of the AE will be documented in the source documents and will be described in the final CSR only if considered relevant by the Investigator.

8.3 Pregnancy

If the Investigator becomes aware of a pregnancy occurring in the partner of a male subject participating in the study up to 90 days after the last dose of study drug of the male subject, the pregnancy should be reported to the Sponsor within 1 working day of obtaining written consent from the pregnant partner. The Investigator may make arrangements for the partner to be counseled by a specialist, to discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the partner should continue untile the outcome of the pregnancy is known.

SERIOUS ADVERSE EVENT CONTACT INFORMATION

In case of a serious adverse event (see Appendix 8.2), the Principal Investigator will send a report within 24 hours of notification to:

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Phone: +49 621 8782 154

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SUMMARY OF CHANGES

The following changes have been introduced in this Version 2.0 of the protocol (dated 01 Jul 2021) and are given as a combination of double underlined and italic text. Deleted text is given as ~~double strikethrough~~.

The rationale for these changes is the change in blood sample volumes and timepoints, and changes in the reporting of metabolite profiling/identification and PK. In addition, contact information of the Client's Clinical Project Manager has been altered.

The changes can be found in:

- *Contact Information*
- *Synopsis*
- *Table 1 - Schedule of Assessments*
- *Section 3.5.1.3 Total Blood Volume: Table 3 - Number and Volume of Blood Samples and Total Blood Volume Collected per Subject*
- *Section 3.5.3.3 Exploratory Variables*
- *Section 3.5.4 Drug Concentration Measurements*
- *Section 3.6.2.1 Pharmacokinetic Evaluation*
- *Section 3.6.2.3 Evaluation of Exploratory Variables*

SYNOPSIS

Study Title

A SINGLE-CENTER, OPEN-LABEL STUDY TO INVESTIGATE MASS BALANCE, EXCRETION PATHWAYS AND METABOLITES AFTER A SINGLE ORAL DOSE OF 500 MG, 3.7 MBq, [^{14}C]BTZ-043 IN HEALTHY MALE VOLUNTEERS

Study Codes

Sponsor code : LMU-IMPH-BTZ-043-03
PRA code : NVS543EC-185431
EudraCT number : 2021-000449-42

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Promotor's contact : Florian Kloss, PhD

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Client's Clinical Project Manager : ~~Flavia Koch, PhD, MSc~~ *Astrid Patzlaff, PhD*

Contract Research Organization and Clinical Site

PRA Health Sciences – Early Development Services, Van Swietenlaan 6, 9728 NZ Groningen, The Netherlands

Principal Investigator

Jan Jaap van Lier, MD

Objectives

Primary : To determine the rates and routes of excretion of [^{14}C]BTZ-043-related radioactivity, including mass balance of total drug-related radioactivity in urine and feces (and vomit, if applicable), following the oral administration of a single 500 mg dose of [^{14}C]BTZ-043 in healthy male volunteers.
: To determine the pharmacokinetics (PK) of total radioactivity in blood and in plasma.
: To characterize the plasma PK of BTZ-043 and main metabolites by liquid chromatography-mass spectrometry (LC-MS), if applicable.
: To characterize the urine concentrations of BTZ-043 and main metabolites by LC-MS, if applicable.

Secondary : To assess the safety and tolerability of a single 500 mg oral dose of BTZ-043 administered to healthy volunteers.

Exploratory : To identify BTZ-043's metabolites in plasma, and to quantify metabolites not measured in the primary objectives by radiometry in order to elucidate key biotransformation pathways and clearance mechanisms of BTZ-043 in humans.

: To identify BTZ-043's metabolites in excreta (urine and feces) and to quantify metabolites not measured in the primary objectives by radiometry in order to elucidate key biotransformation pathways and clearance mechanisms of BTZ-043 in humans.

Design and Treatments

This study is a Phase 1, single-center, open-label study to investigate the absorption, metabolism, and excretion of BTZ-043 after a single oral administration of 500 mg BTZ-043 containing 3.7 MBq of [^{14}C]BTZ-043 in 4 healthy adult male subjects.

A total of 4 evaluable subjects completing all procedures are required. Six (6) subjects will be enrolled in the cohort in order to have 4 evaluable subjects. If the cohort of 4 subjects administered with 500 mg BTZ-043 containing 3.7 MBq of [^{14}C]BTZ-043 leads to less than 4 evaluable subjects, the 2 back-up subjects can be dosed subsequently to ensure that 4 subjects have completed all study procedures and are evaluable. If any of the 4 subjects vomits within 4 hours after study drug administration (approximately 2 times the median T_{max} of BTZ-043), the subject will be replaced. The 2 back-up subjects will stay in the clinical research center until the morning of Day 2.

The study will consist of a screening period (Day -21 to -2), a baseline period (Day -1), a single dose treatment on Day 1 with a minimum of 96 hours (=4 days) post dose in-house observation period (Days -1 up to afternoon Day 5), and a follow-up visit 30 days (±2 days) after the [^{14}C]BTZ-043 dose. The subjects will be admitted to the clinical site on Day -1, at least 17 hours before the administration of [^{14}C]BTZ-043 for baseline evaluation. Subjects will be administered a single 500 mg [^{14}C]BTZ-043 dose as drinking suspension. Subjects will be confined to the clinical site for at least 96 hours following drug administration (ie, afternoon of Day 5). During this time, blood, feces, and urine samples for measurement of [^{14}C]BTZ-043 and metabolites will be collected. The radioactivity excreted in urine and feces as well as radioactivity in blood and plasma should be measured and the data should be communicated daily to Matthias Bader or his designee. The subjects will be released from the clinic approximately 96 hours to 168 hours after dose administration and upon satisfactory recovery of radioactivity (at least 90%) approved by the Sponsor's scientific advisor after consulation of the Sponsor. This will depend on the radioactivity excretion balance as assessed by a normal counting method or real time rapid analysis on site ('quick counting' procedure, utilizing liquid scintillation counter, or sample combustion apparatus) of radioactivity in plasma, urine, and feces accumulated until 96 hours, as feasible. The data has to be available before subjects will be released from the clinic.

The in-house period should be prolonged up to the morning of Day 8 if at least 1 of the following applies:

- Total radioactivity in plasma is higher than 5% of total radioactivity-C_{max} and/or PK parameters for determination of the total radioactivity-elimination phase cannot be determined reliably.
- The mass balance in urine and feces is not complete by Day 5 (ie, <90%).
- The combined urinary and fecal excretion is not ≤1% of the administered dose per 24 hours for 2 consecutive days based on ^{14}C-radioactivity "quick counts" or normal counts, as feasible.

In the event of an incomplete mass balance on the morning of Day 8, subjects may be discharged from the clinical site upon discretion of the Investigator. Subjects with an incomplete mass balance (<90% of dose administered and/or combined urinary and fecal excretion >1% of the administered dose per 24 hours for 2 consecutive days based on ^{14}C-radioactivity "quick counts" or normal counts, as feasible) on Day 8 will be asked to return to the clinical site for one or two 24-hour visits (Day 11-12 and Day 15-16).

In the event of subjects not defecating for 2 days, measures will be taken to ensure defecation (eg, additional fibers, plum juice, dried prunes, and/or sufficient hydration).

Study Schedule

Screening	: Between Day -21 and Day -2.
Treatment period	: From Day -1 (admission) to approximately 96 hours after study drug administration (Day -1 up to afternoon Day 5) with possible extension (Day 6 to Day 8) as well as possible 24-hour visits (Day 11-12, Day 15-16).
Follow-up	: 30 days (±2 days) after the [^{14}C]BTZ-043 dose.

Subjects

4 healthy male subjects

Main Criteria for Inclusion

Age	: 18 years to 55 years, inclusive, at screening
Weight	: 55 to 90 kg, inclusive, at screening
Body mass index	: 18.0 to 29.0 kg/m^2, inclusive, at screening

Study Drug

Active Medication

Active substance	: BTZ-043
Activity	: Inhibition of *Mycobacterium tuberculosis* DprE1
In development for	: Tuberculosis
Dose	: 1 x 100 mL (equivalent to 500 mg BTZ-043)
Strength	: 5 mg/mL BTZ-043 containing 0.037 MBq/mL of [^{14}C]-BTZ-043
Dosage form	: Oral suspension
Manufacturer	: Prepared by pharmacy at PRA

Variables

Safety	: Adverse events, clinical laboratory, vital signs, 12-lead electrocardiogram, physical examination
Pharmacokinetics	: Total radioactivity concentrations in plasma, whole blood, urine, and feces Total radioactivity in plasma and whole blood PK parameters estimated using noncompartmental analysis, as appropriate : C_{max}, t_{max}, $t_{1/2}$, AUC_{inf}, AUC_{0-t}, and whole blood to plasma ratios Concentrations of BTZ-043 and main metabolites in plasma and urine Plasma and urine BTZ-043 PK parameters estimated using noncompartmental analysis, as appropriate: C_{max}, t_{max}, $t_{1/2}$, k_{el}, AUC_{inf}, AUC_{0-t}, CL/F, V_z/F, Ae_{urine}, Fe_{urine}, and CL_r Plasma and urine metabolites PK parameters estimated using noncompartmental analysis, as appropriate: C_{max}, t_{max}, $t_{1/2}$, k_{el}, AUC_{inf}, AUC_{0-t}, Ae_{urine}, and Fe_{urine} Urine and feces (and vomit, if available) radioactivity PK parameters estimated using noncompartmental analysis, as appropriate: Ae_{urine}, Fe_{urine}, Ae_{feces}, Fe_{feces}, and, if available, Ae_{vomit}, and Fe_{vomit}
Exploratory	: Metabolite profiles, metabolite identification, as feasible $\%AUC_{total\ radioactivity}$ of BTZ-043 and metabolites

Statistical Methods

Safety parameters	:	Descriptive statistics
PK parameters	:	Descriptive statistics for all relevant PK parameters: n, number of missing values to be reported (m), geometric mean, geometric coefficient of variation, mean, coefficient of variation, SD, median, minimum, and maximum
Exploratory parameters	:	Descriptive statistics

Table 1 Schedule of Assessments

Visit	Screening	Treatment Period[a]									24-hour Visits[a]				Follow-up
Study Day	**Days -21 to -2**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6[m]**	**Day 7[m]**	**Day 8[m]**	**Day 11[m]**	**Day 12[m]**	**Day 15[m]**	**Day 16[m]**	**Day 31 (±2 days)**
Confinement[a]		X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Ambulatory	X														X
Admission		X									(X)		(X)		
Discharge[b]							X	(X)	(X)	(X)		(X)		(X)	
Informed Consent	X														
Medical History	X														
Demographics	X														
Physical Examination[c]	X	X													X
Body Weight	X	X													
Height and BMI Calculation	X														
Serology (HBsAg, anti-HCV, anti-HIV 1 and 2)	X														
SARS-CoV-2 PCR[d]		X		X							(X)		(X)		
Drug and Alcohol Screen	X	X													
Clinical Laboratory[e]	X	X		X		X									X
Urinalysis	X	X		X		X									X
12-lead ECG[f]	X	X	X	X			X	(X)	(X)	(X)		(X)		(X)	X
Vital Signs[g]	X	X	X	X			X	(X)	(X)	(X)		(X)		(X)	X
Eligibility Check	X	X	X												
Study Drug Administration			X												
Plasma Sampling for BTZ-043 and Main Metabolites[h]			X	X	X										
Whole Blood Sampling for Radioactivity[i]			X	X	X	X	X	(X)	(X)	(X)	(X)		(X)		
Plasma Sampling for Total Radioactivity and Metabolite Profiling[i]			X	X	X	X	X	(X)	(X)	(X)	(X)		(X)		

Visit	Screening	Treatment Period[a]									24-hour Visits[a]				Follow-up
Study Day	**Days -21 to -2**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6[m]**	**Day 7[m]**	**Day 8[m]**	**Day 11[m]**	**Day 12[m]**	**Day 15[m]**	**Day 16[m]**	**Day 31 (±2 days)**
Urine Collection and Pooling for BTZ-043 and Main Metabolites, Total Radioactivity, and Metabolite Profiling[j]		X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Feces Collection for Total Radioactivity and Metabolite Profiling[k]	X	X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Vomit Collection for Total Radioactivity, if available[l]			X												
Previous and Concomitant Medication	X	X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X
AE Monitoring		X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X

AE=adverse event; BMI=body mass index; ECG=electrocardiogram; HBsAg=hepatitis B surface antigen; HCV=hepatitis C virus; PCR=polymerase chain reaction; PK=pharmacokinetic(s)

a Subjects will be in the clinic from Day -1 (predose) until Day 5 with possible extension (Day 6 to Day 8) as well as possible 24-hour visits (Day 11-12, Day 15-16). The 2 back-up subjects will stay in the clinic until the morning of Day 2.

b Subjects will be released from the clinic 96 hours after dose administration and upon satisfactory recovery of radioactivity.

c Complete physical examinations will be conducted at screening, Day -1, and follow-up/early termination. Symptom driven physical examinations may be conducted at any time, per the Investigator's discretion.

d Sampling of nasal and throat mucosal cells. If deemed necessary, additional tests may be conducted during the study. The nonclinical project manager will be informed immediately upon availability of Day 11 results, as this will qualify the feces samples for metabolite profiling with regard to potential COVID-19 infectivity.

e Clinical laboratory tests (including clinical chemistry, hematology, and urinalysis): at screening; on Day -1 (admission), Day 2, and Day 4; and at follow-up.

f 12-lead ECG: at screening; Day -1, and 1 hour, 4 hours, and 24 hours postdose; at discharge; and at follow-up.

g Vital signs (supine systolic and diastolic blood pressure, pulse, body temperature, and respiratory rate): at screening; at admission; at predose and at 1 hour, 4 hours, and 24 hours postdose; at discharge; and at follow-up.

h Plasma sampling for PK of BTZ-043 and main metabolites in plasma: at predose and 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16, 24, 36, and 48 hours postdose.

i Total radioactivity in whole blood and plasma~~, and metabolite profiling in plasma~~: at predose and 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16, 24, 36, 48, 72, and 96 hours postdose (in case total radioactivity recovered is still <90%: in addition 120, 144, and 168 hours postdose and at further 24-hour visits [264 and 360 hours postdose] until study completion). *Metabolite profiling in plasma: at predose and 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16, 24, 36, 48, 72, and 96 hours postdose (in case total radioactivity recovered is still <90%: in addition 120, 144, and 168 hours postdose.*

j Urine collection and pooling for BTZ-043 and main metabolites, total radioactivity, and metabolite profiling: at predose (within 12 hours prior to dosing) and over 0-24, 24-48, 48-72, and 72-96 hours postdose collection intervals (in case total radioactivity recovered is still <90%, in addition 96-120, 120-144, and 144-168 hours postdose and at further 24-hour intervals [Day 11-12 and Day 15-16] until study completion).

k Feces collection for total radioactivity and metabolite profiling; predose feces collection to be collected at any time within 48 hours prior to study drug administration (at home or in the clinic). After administration of BTZ-043, feces will be collected in 24 hour collection intervals up to 96 hours postdose (in case total radioactivity recovered is still <90%: in addition 120, 144, and 168 hours postdose and at further 24-hour intervals [Day 11-12 and Day 15-16] until study completion).

l If produced, vomit will be collected up to 8 hours after the administration of the study drug for total radioactivity determination.

m Day 6-8 procedures will only be conducted in case that the recovery of radioactivity was not satisfactory until Day 5. 24-hour visits procedures will only be conducted in case that the recovery of radioactivity was not satisfactory until Day 8. For the 24-hour visits (if applicable): feces collection to be collected at home within 48 hours prior to admission.

TABLE OF CONTENTS

TABLE OF TABLES

LIST OF ABBREVIATIONS

AME	absorption, metabolism, and excretion
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BMI	body mass index
CA	Competent Authority
CCMO	Centrale Commissie Mensgebonden Onderzoek (Central Committee on Research Involving Human Subjects)
CHMP	Committee for Medicinal Products for Human Use
GMP	Good Manufacturing Practice
CSP	clinical study protocol
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CTD	Clinical Trial Directive
DprE1	decaprenylphosphoryl-β-D-ribose-2'-epimerase
ECG	electrocardiogram
eCRF	electronic case report form
EDS	Early Development Services
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HED	human equivalent dose
HKI	Hans Knöll Institut
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
MEB	Medicine Evaluation Board
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no observed adverse effect level
PCR	polymerase chain reaction
PK	pharmacokinetic(s)
PRA	PRA Health Sciences
SAE	serious adverse event
SAP	statistical analysis plan
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
WMA	World Medical Association
WMO	Wet medisch-wetenschappelijk onderzoek met mensen (medical research involving human subjects act)

Note: Definitions of pharmacokinetic (PK) parameters are provided in Section 3.5.3.

1. INTRODUCTION

1.1 Background

BTZ-043 is a promising new antibiotic for the treatment of tuberculosis. Its mechanism of action is based on a reduction of BTZ-043 to a transient nitroso moiety, which then reacts with an essential cysteine residue in the enzyme decaprenylphosphoryl-β-D-ribose-2'-epimerase (DprE1), forming a semimercaptal as covalent adduct. DprE1 itself is required for the biosynthesis of decaprenylphosphoryl-β-D-arabinose, an essential component for the cell wall assembly of mycobacteria. Formation of the covalent adduct between BTZ-043 and DprE1 results in inhibition of cell wall biosynthesis and loss of viability of *M. tuberculosis*. Due to this mechanism BTZ-043 is highly selective for mycobacteria species (and some other members of Actinomycetales), but does not affect the gut flora.[1]

In 2018 a Phase 1a study entitled “A Randomized, Double blind, Placebo-controlled, Single Ascending Dose Study to Evaluate Safety, Tolerability, and Pharmacokinetics (PK) of Single Doses of BTZ-043 in Healthy Adult Volunteers” (LMU-IMPH-BTZ-043-01) was conducted in humans and currently a Phase 1a/2b study entitled “A Prospective Phase 1b/2a, Active-controlled, Randomized, Open-label Study to Evaluate the Safety, Tolerability, Extended Early Bactericidal Activity and PK of Multiple Oral Doses of BTZ-043 Tablets in Subjects With Newly Diagnosed, Uncomplicated, Smear-positive, Drug-susceptible Pulmonary Tuberculosis” (PanACEA-BTZ-043-02) is ongoing. This human absorption, metabolism, and excretion study is conducted during Phase 2 proof of concept studies and before a Phase 3 study is initiated.

1.1.1 Nonclinical Summary

Safety

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Pharmacokinetics

[redacted]

[redacted]

1.1.2 Clinical Summary

Safety

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted].

[redacted].

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

1.2 Risk-benefit Assessment

There is no expected clinical benefit for the healthy subjects who will participate in this study. The information obtained in this study can be used for the further clinical development of BTZ-043. As depicted in Table 2, BTZ-043 has been well tolerated in much higher exposures as proposed in this study.

Overall, on the basis of the available nonclinical and clinical data, the risk-benefit profile of BTZ-043 is judged acceptable for the proposed clinical study.

The risk-benefit assessment for the subjects receiving BTZ-043 remains unchanged in relation to the corona virus disease-19 (COVID-19) pandemic as available clinical and nonclinical results do not suggest that administration of BTZ-043 will lead to suppression or modulation of the immune system. In addition, the mode of action does not appear to have a substantial effect on the respiratory or cardiovascular system critically affected by a SARS-CoV-2 infection. As the subjects to be included in this study are in general young to middle aged without major comorbidities, the study population is not considered to be a high-risk population for serious COVID-19 disease. Only persons with a negative SARS-CoV-2 test at admission to the clinical research center will be allowed to participate in the study. In addition, all appropriate measures to prevent SARS-CoV-2 infection during the study will be taken as detailed in Section 3.2.1.

1.3 Study Rationale

This study will determine the disposition and metabolism of BTZ-043 in humans after oral administration. The use of radiolabeled molecules (usually [^{14}C]) is a common method to ascertain information on the elimination routes and metabolic fate of a compound at an early stage of development. This evaluation will provide an estimate of PK parameters, the routes and rates of elimination of radioactivity, and identification of metabolites and metabolic pathways. These data will be compared with similar data obtained from nonclinical studies, both from PK and metabolic points of view. The rationale for the proposed dose can be found in Section 3.4.4.

2. OBJECTIVES

2.1 Primary

- To determine the rates and routes of excretion of [^{14}C]BTZ-043-related radioactivity, including mass balance of total drug-related radioactivity in urine and feces (and vomit, if applicable), following the oral administration of a single 500 mg dose of [^{14}C]BTZ-043 in healthy male volunteers.
- To determine the PK of total radioactivity in whole blood and in plasma.
- To characterize the plasma PK of BTZ-043 and main metabolites by liquid chromatography-mass spectrometry (LC-MS), if applicable.
- To characterize the urine concentrations of BTZ-043 and main metabolites by LC-MS, if applicable.

2.2 Secondary

- To assess the safety and tolerability of a single 500 mg oral dose of BTZ-043 administered to healthy male volunteers.

2.3 Exploratory

- To identify BTZ-043's metabolites in plasma, and to quantify metabolites not measured in the primary objectives by radiometry in order to elucidate key biotransformation pathways and clearance mechanisms of BTZ-043 in humans.
- To identify BTZ-043's metabolites in excreta (urine and feces) and to quantify metabolites not measured in the primary objectives by radiometry in order to elucidate key biotransformation pathways and clearance mechanisms of BTZ-043 in humans.

3. INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

3.1.1 Type of Study

This will be a Phase 1, single-center, open-label, absorption, metabolism, and excretion (AME) study in 4 healthy adult male subjects.

3.1.2 Screening Period

Subjects will report to the medical screening facility/clinical site for the eligibility screening (see Section 3.3 for inclusion and exclusion criteria) within 3 weeks prior to drug administration.

Subjects will sign the study-specific informed consent form (ICF) prior to any study-specific screening procedures being performed. The written informed consent will be obtained for all subjects, regardless of their eligibility for the study; the signed ICFs will be retained and archived at PRA Health Sciences (PRA) and a copy will be provided to the subject.

Eligibility screening will consist of the assessments as presented in the schedule of assessments (Table 1).

3.1.3 Treatment Period

Subjects will be admitted to the clinical research center on Day -1, which is the day prior to Day 1, the day of drug administration.

Subjects will receive a single oral dose of 500 mg BTZ-043 containing 3.7 MBq of [^{14}C]BTZ-043 on Day 1. Subjects will be discharged from the clinical research center on Day 5 (approximately 96 hours after drug administration) upon satisfactory recovery of radioactivity. If the criteria for discharge are not met on Day 5, subjects will be required to remain confined for a maximum of 3 additional days (Days 6 to 8) until the criteria are met (daily check by quick counts or normal counts, as feasible). The in-house period should be prolonged up to the morning of Day 8 if at least 1 of the following applies:

- Total radioactivity in plasma is higher than 5% of radioactivity-C_{max} and/or PK parameters for determination of the total radioactivity-elimination phase cannot be determined reliably.
- The mass balance in urine and feces is not complete by Day 5 (ie, >90%).
- The combined urinary and fecal excretion is not ≤1% of the administered dose per 24 hours for 2 consecutive days based on ^{14}C-radioactivity “quick counts” or normal counts, as feasible.

If the criteria for discharge are still not met on Day 8, subjects may be discharged from the clinical site upon discretion of the Investigator. To ensure that adequate consideration is given to the protection of the subject’s interests, subjects with an incomplete mass balance on Day 8 will be asked to return to the clinical site for one or two 24-hour visits (Day 11-12 and Day 15-16).

On the day of study drug administation (Day 1), an FDA-recommended high-fat (or a standardized breakfast, if applicable, as outlined in Section 3.4.4 and Section 3.4.7) with calculated caloric content will be provided.

Assessments during the treatment period will be performed as presented in the schedule of assessments (Table 1).

3.1.4 Follow-up

The follow-up assessments will be performed 30 days (±2 days) after the [^{14}C]BTZ-043 dose.

Assessments during follow-up will be performed as presented in the schedule of assessments (Table 1).

3.2 Discussion of Study Design

The current design is commonly used for AME studies and in accordance with the current EMA "Guideline on the investigation of drug interactions". [2] Since the present study is a descriptive study aimed at assessing excretion routes of BTZ-043 and evaluating mass balance, a formal statistical sample size calculation was not performed. A sample size of 4 subjects is a minimum, accepted number of subjects for AME studies and is considered sufficient to achieve the study objectives. Early-termination subjects may be replaced if the total number of completers drops below 4, after mutual agreement between the Sponsor and PRA.

3.2.1 COVID-19 Risk Mitigation

This study will be conducted in accordance with guidance from the Central Committee on Research Involving Human Subjects (CCMO [Centrale Commissie Mensgebonden Onderzoek], ie, the Dutch competent authority [CA]) on conducting Phase 1 trials in clinical research centers in The Netherlands during the COVID-19 pandemic.

During the entire study, the clinical research center will implement all recommendations issued by the Dutch government, including specific guidelines related to clinical research executed in clinical research centers with respect to minimizing the risk of disease spreading (eg, social distancing, disinfection, hygiene, and wearing of personal protection equipment by study staff). Details on specific procedures are described in the site specific manual.

In cases where subjects are not able to attend study visits due to an infection with SARS-CoV-2, the Investigator will discuss with the Sponsor potential mitigation approaches (including, but not limited to, extending the visit window, conducting evaluations via video link or phone call, allowing for safety procedures to be conducted at a local facility). The rationale (eg, the specific limitation imposed by the SARS-CoV-2 infection that led to the inability to perform the protocol-specified assessment) and outcome of the discussion will be documented in the electronic case report form (eCRF).

In addition, the following containment measures will be taken during the study:

- Polymerase chain reaction (PCR) testing for SARS-CoV-2 will be performed at the time points indicated in Table 1.
- A subject should not be admitted if there was any contact with a person who tested positive for SARS-CoV-2 or a COVID-19 patient within the last 2 weeks prior to admission to the clinical research center.
- If a subject is tested to be SARS-CoV-2 positive on Day -1, the subject will be excluded from participation with reference to Exclusion Criterion 13, and referred for treatment.
- Physical examinations will be limited.
- If a subject becomes ill and/or is tested to be SARS-CoV-2 positive after the administration of study treatment, study assessments will be stopped (see also Section 3.3.3.1). A safety follow-up can be done if deemed necessary by the Investigator. The subject will be kept in-house in quarantine with appropriate medical intervention until complete elimination of the study compound or will be asked to quarantine at home according to guidelines of the Dutch government.

3.3 Selection of Study Population

3.3.1 Inclusion Criteria

Note: Restrictions that apply to the period after admission are described in Section 3.4.9, except when they concern a statement of willingness.

The following inclusion criteria must be met for a subject to be eligible for inclusion in the study:

1. Sex : male
2. Age : 18 years to 55 years, inclusive, at screening.
3. Body mass index (BMI) : 18.0 to 29.0 kg/m^2, inclusive, at screening.
4. Weight : 55 to 90 kg, inclusive, at screening.
5. Status : healthy subjects.
6. Male subjects, if not surgically sterilized, must agree to use adequate contraception and not donate sperm from admission to the clinical research center until 90 days after the follow-up visit. Adequate contraception for the male subject (and his female partner, if she is of childbearing potential) is defined as using hormonal contraceptives or an intrauterine device combined with at least 1 of the following forms of contraception: a diaphragm, a cervical cap, or a condom. Total abstinence, in accordance with the lifestyle of the subject, is also acceptable.
7. All prescribed medication must have been stopped at least 30 days prior to admission to the clinical research center.
8. All over-the-counter medications, vitamin preparations (especially vitamin C), other food supplements, and herbal medications (eg, St. John's wort) must have been stopped at least 14 days prior to admission to the clinical research center. An exception is made for paracetamol, which is allowed up to 48 hours prior to study drug administration.
9. No vaccination within 14 days prior to study drug administration.

10. Ability and willingness to abstain from alcohol from 48 hours (2 days) prior to screening and admission to the clinical research center.
11. Ability and willingness to abstain from methylxanthine-containing beverages or food (coffee, tea, cola, chocolate, and energy drinks), grapefruit (juice), corn (whole corn kernels and popcorn), cruciferous vegetables, and bitter oranges from 48 hours (2 days) prior to admission to the clinical research center.
12. Good physical and mental health on the basis of medical history, physical examination, clinical laboratory, ECG, and vital signs, as judged by the Investigator.
13. Willing and able to sign the ICF.

3.3.2 Exclusion Criteria

Note: Restrictions that apply to the period after admission are described in Section 3.4.9, except when they concern a statement of willingness.

A subject who meets any of the following exclusion criteria will not be eligible for inclusion in the study:

1. Participation in another study with a radiation burden of >0.1 mSv and ≤1 mSv in the period of 1 year prior to screening; a radiation burden of >1.1 mSv and ≤2 mSv in the period of 2 years prior to screening; a radiation burden of >2.1 mSv and ≤3 mSv in the period of 3 years prior to screening, etc.
2. Exposure to radiation for diagnostic reasons (except dental X-rays and plain X-rays of thorax and bony skeleton [excluding spinal column]), or during work within 1 year prior to drug administration.
3. Irregular defecation pattern (less than once per day on average).
4. Employee of PRA, Nuvisan, or the Sponsor.
5. History of relevant drug and/or food allergies.
6. Using tobacco products within 60 days prior to drug administration.
7. History of alcohol abuse or drug addiction (including soft drugs like cannabis products).
8. Positive drug and alcohol screen (opiates, methadone, cocaine, amphetamines [including ecstasy], cannabinoids, barbiturates, benzodiazepines, gamma-hydroxybutyric acid, tricyclic antidepressants, and alcohol) at screening or admission to the clinical research center.
9. Average intake of more than 24 grams of alcohol per day.
10. Positive screen for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibodies, or HIV 1 and 2 antibodies.
11. Participation in a drug study within 30 days prior to drug administration in the current study. Participation in more than 4 drug studies in the 12 months prior to drug administration in the current study.
12. Donation or loss of more than 450 mL of blood within 60 days prior to drug administration. Donation or loss of more than 1.5 liters of blood in the 10 months prior to drug administration in the current study.
13. Significant and/or acute illness within 5 days prior to drug administration that may impact safety assessments, in the opinion of the Investigator.

14. Unwillingness to consume the Food and Drug Administration (FDA)-recommended high-fat breakfast.
15. Unsuitable veins for infusion or blood sampling.
16. Positive nasopharyngeal PCR test for SARS-CoV-2 on Day -1.

Please note that subjects should refrain from consumption of any foods containing poppy seeds, corn (whole corn kernels and popcorn), cruciferous vegetables, and bitter oranges within 48 hours (2 days) prior to screening and admission to the clinical research center to avoid false positive drug screen results, difficult feces texture, or induction of drug metabolizing enzymes. In addition, they should refrain from strenuous exercise within 96 hours (4 days) prior to screening and admission as this could result in abnormal clinical laboratory values.

3.3.3 Removal of Subjects from Assessment

Participation in the study is strictly voluntary. A subject has the right to withdraw from the study at any time for any reason.

The Investigator has the right to terminate participation of a subject for any of the following reasons: difficulties in obtaining blood samples, violation of the protocol, severe AEs or SAEs, or for any other reason relating to the subject's safety or integrity of the study data.

If a subject is withdrawn from the study, Nuvisan and LMU Klinikum will be informed immediately. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned, or the condition has stabilized with no more change being likely.

After signing informed consent, subjects who drop out or withdraw for any reason without completing all screening evaluations successfully as well as subjects who drop out or withdraw prior to the first dose of study drug will be considered screening failures.

A subject who is withdrawn or voluntarily withdraws from the study for any reason, whether related to the study drug or not, after having received the study drug, will be considered an early-termination subject. If a subject is withdrawn from the study, the early-termination subject will be replaced after mutual agreement between the Sponsor and PRA if the number of evaluable subjects drops below 4 (with a maximum of 2 replacements).

The decision regarding the replacement of subjects will be documented.

PRA will make every effort to ensure that early-termination subjects who have received the study drug complete the early-termination assessments/safety follow-up assessments.

Early-termination subjects may be replaced if the total number of completers drops below 4, after mutual agreement between Nuvisan, LMU Klinikum, and PRA.

3.3.3.1 Stopping Rules for Individual Subjects

In case of a positive SARS-CoV-2 virus PCR test or evidence for COVID-19 during the study, the subject will be withdrawn from the study. The subject will be isolated from other study participants and referred for treatment. The subject will be followed up in quarantine in the clinical research center until complete elimination of the study compound or will be asked to quarantine at home according to guidelines of the Dutch government.

3.4 Treatments

3.4.1 Treatments Administered

Subjects will receive a single oral dose of 500 mg BTZ-043 containing 3.7 MBq of [^{14}C]BTZ-043 on Day 1.

3.4.2 Identity of Investigational Product

Active Medication

Active substance	:	BTZ-043
Activity	:	Inhibition of *M. tuberculosis* DprE1
In development for	:	Tuberculosis
Dose	:	1 x 100 mL (equivalent to 500 mg BTZ-043)
Strength	:	5 mg/mL BTZ-043 containing 0.037 MBq of [^{14}C]BTZ-043
Dosage form	:	Oral suspension
Manufacturer	:	Sourced by pharmacy at PRA

The active pharmaceutical ingredient will be provided by the Promotor.

[^{14}C]BTZ-043 is a drug under development and not approved yet. It will be manufactured and analysed according to Good Manufacturing Practice (GMP) requirements. A GMP certificate of the manufacturer of the [^{14}C]BTZ-043 suspension and an Investigator's Brochure that summarizes preclinical and clinical data with respect to BTZ-043 are in place. A certificate of analysis and a certificate of conformity will be provided by PRA prior to clinical part.

For details concerning drug storage and drug accountability see Appendix 8.1.

3.4.3 Method of Assigning Subjects to Treatment Groups

After obtaining informed consent, subjects will receive a screening number and will be screened according to the inclusion and exclusion criteria. Subjects who have met all eligibility criteria will receive a subject number upon inclusion in the study (Subject Numbers 001-004). They will receive the subject number just prior to dosing. The subject number will ensure identification throughout the study after study drug administration. Replacement subjects will receive the number of the subject to be

replaced, increased by 100 (eg, 101 replacement number for Subject Number 001), and will be administered the same treatment.

After signing informed consent, subjects who drop out or withdraw for any reason without completing all screening evaluations successfully as well as subjects who drop out or withdraw prior to the first dose of study drug will be considered screening failures. Such subjects, and also subjects who are eligible for inclusion in the study but do not receive the study drug, will not receive a subject number, and only applicable data will be entered in the eCRFs.

3.4.4 Selection of Doses and Meals in the Study

The targeted dose of 500 mg is considered a clinically relevant dose for the human AME study (Table 2) since this exposure achieves a maximal bactericidal effect and has shown to be tolerable in the 14-day Phase 1b study. It is proposed that the active pharmaceutical ingredient is taken after a high-fat breakfast (see Section 3.4.7), as absorption is then much improved. As shown in Table 2, the expected exposure is well below the maximum and well tolerated exposure achieved in the Phase 1b study. In the unexpected case that the currently conducted Phase 2a study with 250 mg, 500 mg, and 1000 mg BTZ-043 administered after a high-fat breakfast shows any dose-related safety alert, the study team and the Sponsor will take measures to reduce the exposure. Reduction of exposure will be achieved by adapting the food intake (up to fasting) before the administration of [^{14}C]BTZ-043. This protocol describes the drug administration of 500 mg [^{14}C]BTZ-043 after a high-fat breakfast as default. However, the study team and the Sponsor will decide whether a reduction in food intake is necessary to reduce the exposure to [^{14}C]BTZ-043.

In case it is indeed decided to administer 500 mg [^{14}C]BTZ-043 after a standardized breakfast or in the fasted state rather than after a high-fat breakfast as planned, this decision and the associated rationale will be laid down in a Note to File, to be signed by the Sponsor and the PI, and to be submitted to the IEC for information only.

The dose of ^{14}C (approximately 3.7 MBq) to be administered was chosen to ensure successful profiling and identification of metabolites.

In the present study, the estimated effective radiation burden after a single oral radioactivity dose of 3.7 MBq is approximately 0.16 mSv. [3] For biomedical investigations in small groups of human volunteers, an effective dose of 0.1 to 1.0 mSv is considered acceptable.

3.4.5 Radiation Safety of Clinical and Laboratory Staff

It is expected that the clinical and laboratory staff handling the radioactive material and samples will not be exposed to any relevant radiation and associated health risk. The [^{14}C] isotope emits low energy beta particles, which are not able to penetrate the dose containers or sample container walls. Therefore, there will be no “external” exposure from sample handling. The samples from the participants after

radiolabeled dose administration will contain low levels of radioactivity. The radioactive dose of 3.7 MBq [^{14}C] per subject is below the legal handling limit, above which a special laboratory and special procedures for handling radioactivity would be required. Therefore, the staff can handle the study samples like any other non-radioactive biological material. Common practice of safe laboratory technique and hygiene will be exercised in handling all biological materials from this study.

3.4.6 Timing of Doses in the Study

On Day 1, the study drug will be administered with the subject in the upright position. The study drug will be administered to subjects between 08:00 and 11:00 in the morning. After an overnight fast of at least 10 hours, subjects will receive an FDA-recommended high-fat breakfast (or a standardized breakfast, if applicable as outlined in Section 3.4.4) that will have to be finished within 20 minutes. The entire breakfast must be consumed by the subjects. The actual consumed food will be documented.

Dosing will occur at 30 minutes after the start of the high-fat breakfast (or the standardized breakfast, if applicable, or dosing will occur in a fasted state, if applicable as outlined in Section 3.4.4).

The study drug will be given as an oral suspension of 100 mL, which will be provided in an amber glass bottle from which the subjects can drink. Thereafter the bottle will be rinsed twice with 70 mL of water, and the subjects will be required to drink the bottle contents after rinsing. Thereafter, the subjects are required to drink additional non-carbonated water until they have ingested a total volume of 380 mL.

Following drug administration, subjects will fast for a period of 4 hours until lunch. During fasting, no fluids are allowed except water; however, water is not allowed from 1 hour predose until 1 hour postdose (apart from the water taken with the dose).

Subjects will not lie down for 4 hours after drug administration, except when required for assessments that need to be performed.

3.4.7 Meals During the Study

A fasting period of at least 4 hours is required before obtaining clinical laboratory blood samples at the time points indicated in the schedule of assessments (Table 1).

An FDA-recommended high-fat (or a standardized breakfast, if applicable, as outlined in Section 3.4.4) with calculated caloric content will be provided on Day 1.

The FDA-recommended high-fat breakfast of 918 kcal consists of:

- 2 fried eggs (in 15 g butter/margarine) (approximately 100 g)
- 1 portion of bacon (40 g) (or brie 60+ for vegetarians)
- 1 portion of fried potatoes (115 g)
- 2 slices of (toasted) (wheat) bread with 15 g margarine
- 1 glass of whole milk (240 mL)

The total of 918 kcal (vegetarian version 915 kcal) can be broken down as follows:

- 39 g protein = 156 kcal
- 59 g fat = 527 kcal
- 59 g carbohydrates = 235 kcal

The standardized breakfast of 572-755 kcal (depending on choices made) consists of:

- 3 slices of whole-wheat bread
- 1 Dutch rusk
- 20 g halvarine/margarine
- 1 portion of cheese/old cheese/cheese spread
- 1 portion of ham/chicken breast/smoked meat/spreadable liver sausage/baloney/filet American/salami (or cheese, cheese spread, or peanut butter for vegetarians)
- 2 portions of jam or other sweet spread
- 2 mugs of decaffeinated coffee/tea (cream and sugar optional, max 2 of both)

The total of 572-755 kcal (vegetarian version 589-805 kcal) can be broken down as follows:

- 18-19 g protein = 71-75 kcal
- 26-32 g fat = 233-292 kcal
- 67-97 g carbohydrates = 268-388 kcal

Other meals and snacks (such as decaffeinated coffee, herbal tea, fruit, and biscuits) will be provided according to PRA standard operating procedures (SOPs), taking into account the restrictions as described in Section 3.4.9 and what has been described in Section 3.4.6.

3.4.8 Blinding

This is an open-label study.

3.4.9 Concomitant Medication and Other Restrictions During the Study

Note: Restrictions that apply to the period before admission are described in Section 3.3.1 and Section 3.3.2.

The use of all prescribed medication is not allowed from admission to the clinical research center until follow-up. The use of all over-the-counter medications, vaccines, vitamin preparations (especially vitamin C), other food supplements, and herbal medications (eg, St. John's wort) is not allowed from admission to the clinical research center until follow-up. An exception is made for paracetamol: from admission onwards, the Investigator may permit a limited amount of paracetamol for the treatment of headache or any other pain. Other medication to treat AEs may only be prescribed if deemed necessary by the Investigator. If medication is used, the name of the drug, the dose, and dosage regimen will be recorded in the eCRF.

The use of methylxanthine-containing beverages or food (coffee, tea, cola, chocolate, and energy drinks), grapefruit (juice), kiwi, and tobacco products is not allowed during the stay in the clinical research center.

The use of alcohol is not allowed during the stay in the clinical research center and within 48 hours (2 days) prior to the 24-hour return visits (Days 11-12 and 15-16, as needed) and prior to follow-up.

Strenuous exercise is not allowed within 96 hours (4 days) prior to admission and follow-up. Strenuous exercise is also not allowed during the stay in the clinical research center.

Subjects should not consume any foods containing poppy seeds, corn (whole corn kernels and popcorn), cruciferous vegetables, and bitter oranges within 48 hours (2 days) prior to admission to the clinical research center as this could cause a false positive drug screen result.

Male subjects, if not surgically sterilized, are required to use adequate contraception (see Inclusion Criterion 6) and not donate sperm from admission to the clinical research center until 90 days after the follow-up visit.

Subjects must not donate blood during the study until the follow-up visit (other than the blood sampling planned for this study).

3.4.10 Treatment Compliance

Study drug will be administered in the clinical research center. To ensure treatment compliance, administration of the study drug will be supervised by the Investigator or an authorized designee. Compliance will be further confirmed by bioanalytical assessment of BTZ-043 and total radioactivity in plasma and urine samples (see Section 3.5.4).

The exact times of study drug administration and the number of units administered will be recorded in the eCRF.

3.5 Pharmacokinetic, Safety, and Exploratory Measurements and Variables

3.5.1 Pharmacokinetic and Safety Measurements Assessed and Schedule of Assessments

A schedule of assessments is presented in Table 1.

3.5.1.1 Pharmacokinetic Measurements

3.5.1.1.1 Blood Sampling

At the time points defined in the schedule of assessments (Table 1), blood samples will be taken for the analysis of BTZ-043 and metabolites in plasma samples, total radioactivity in whole blood and plasma samples, and metabolite profiling and identification in plasma samples.

The blood samples will be taken via an indwelling iv catheter or by direct venipuncture. The exact times of blood sampling will be recorded in the eCRF.

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA and Nuvisan.

3.5.1.1.2 Urine Collection

During the intervals defined in the schedule of assessments, urine will be collected for the analysis of BTZ-043 and metabolites, for total radioactivity, for quick counts or normal counts of total radioactivity (as feasible), and for metabolite profiling and identification. The subjects will be instructed to empty their bladders completely before drug administration and at the end of each collection interval. A baseline urine sample will be collected within 12 hours prior to drug administration. The exact times of urine collection and the urine weight of the entire interval will be recorded in the eCRF.

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA and Nuvisan.

3.5.1.1.3 Feces Collection

During the intervals defined in the schedule of assessments, all fecal excretions will be collected for the analysis of total radioactivity, for quick or normal counts of total radioactivity (as feasible), and for metabolite profiling and identification. A blank fecal sample will be collected within 48 hours prior to study drug administration. The exact times of feces collection and the fecal weight will be recorded in the eCRF.

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA and Nuvisan.

3.5.1.1.4 Vomit Collection

If a subject vomits within the time period from drug administration up to 8 hours after the administration of the study drug, the vomit should, if possible, be collected. Any collected vomit will be included in the total radioactivity measurements and in the determination of the mass balance. The exact times of vomit production will be recorded in the eCRF.

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA and Nuvisan.

3.5.1.2 Safety and Tolerability Measurements

Safety and tolerability assessments will consist of AEs, clinical laboratory, vital signs, 12-lead ECG, and physical examination. Assessments will be performed in accordance with the schedule of assessments.

3.5.1.2.1 Adverse Events

AEs will be recorded from signing the ICF until completion of the follow-up visit. Any clinically significant observations, as determined by the Investigator, in results of clinical laboratory, 12-lead ECGs, vital signs, or physical examinations will be recorded as AEs.

A TEAE is defined as any event not present prior to administration of the study drug or any event already present that worsens in either severity or frequency following exposure to the study drug.

An AE that occurs prior to administration of the study drug will be considered a pretreatment AE.

At several time points before and after drug administration, subjects will be asked nonleading questions to determine the occurrence of AEs. Subjects will be asked in general terms about any AEs at regular intervals during the study. In addition, all AEs reported spontaneously during the course of the study will be recorded. All answers will be interpreted by the Investigator using the Medical Dictionary for Regulatory Activities (MedDRA; most recent version) for AEs and will be recorded in the eCRF as reported terms.

The severity of the AEs will be rated as mild, moderate, or severe; the relationship between the AEs and the study drug will be indicated as related or not related. Details on rating the severity of AEs and relationship to study treatment are given in Appendix 8.2.

Pregnancy of female partners of male subjects will be monitored along with follow-up, if warranted (see Appendix 8.3).

3.5.1.2.2 Clinical Laboratory

Blood and urine samples for clinical laboratory assessments will be collected according to PRA SOPs.
The following parameters will be measured:

- Clinical chemistry (serum quantitatively):
  total bilirubin, alkaline phosphatase, gamma glutamyl transferase, AST, ALT, lactate dehydrogenase, creatinine, urea, total protein, glucose, inorganic phosphate, sodium, potassium, calcium, and chloride.
- Hematology (blood quantitatively):
  leukocytes, erythrocytes, hemoglobin, hematocrit, thrombocytes, absolute partial automated differentiation (lymphocytes, monocytes, eosinophils, basophils, and neutrophils), mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.
- Urinalysis (urine qualitatively):
  hemoglobin, urobilinogen, ketones, glucose, protein.
- Serology:
  HBsAg, HCV antibodies, and HIV 1 and 2 antibodies.

- Drug and alcohol screen:
  opiates, methadone, cocaine, gamma hydroxybutyric acid, amphetamines (including ecstasy), cannabinoids, barbiturates, benzodiazepines, tricyclic antidepressants, nicotine metabolites, and alcohol.

Nasal and throat mucosal cell samples will be collected according to PRA work instructions detailed in the site specific manual. The samples will be tested for SARS-CoV-2 virus using PCR tests. The nonclinical project manager will be informed immediately upon availability of Day 11 results, as this will qualify the feces samples for metabolite profiling with regard to potential COVID-19 infectivity.

In case of unexplained or unexpected clinical laboratory test values, the tests will be repeated as soon as possible and followed up until the results have returned to the normal range and/or an adequate explanation for the abnormality is found. The clinical laboratory will clearly mark all laboratory test values that are outside the normal range, and the Investigator will indicate which of these deviations are clinically significant. Clinically significant laboratory result deviations will be recorded as AEs, and the relationship to the treatment will be indicated (see also Appendix 8.2).

Details on sample collection, handling, storage, and shipping will be described in the laboratory manual prepared by PRA.

3.5.1.2.3 Vital Signs

Systolic and diastolic blood pressure and pulse will be recorded after the subject has been resting for at least 5 minutes in the supine position. These assessments will be made using an automated device whenever possible. Body temperature and respiratory rate will also be measured.

3.5.1.2.4 Electrocardiogram

A standard 12-lead ECG will be recorded after the subject has been resting for at least 5 minutes in the supine position. The ECG will be recorded using an ECG machine equipped with computer-based interval measurements. The following ECG parameters will be recorded: heart rate, PR-interval, QRS-duration, QT-interval, QTc-interval (Fridericia's), and the interpretation of the ECG profile by the Investigator.

3.5.1.2.5 Physical Examination

Physical examination will be performed according to PRA SOPs. In addition, body weight and height will be measured according to PRA SOPs.

3.5.1.3 Total of Blood Volume

Table 3 presents the number and volume of blood samples and the total volume of blood that will be collected throughout the study per subject.

If deemed necessary by the Investigator or the Sponsor, the number and/or volume of blood samples per assessment may be increased, as long as the total volume of blood

drawn for a subject does not surpass 500 mL (except when extra blood samples need to be taken for safety reasons).

Table 3 Number and Volume of Blood Samples and Total Blood Volume Collected per Subject

Assessment	Maximum # Samples	Volume of Blood per Sample (mL)	Total Volume of Blood (mL)
Pharmacokinetics - BTZ-043 and metabolites in Plasma - Total Radioactivity in Plasma - Total Radioactivity in Whole Blood - Metabolite Profiling in Plasma	14 21 21 ~~21~~19	~~4~~2 ~~2~~4 1 ~~12~~15	~~56~~28 ~~42~~84 21 ~~252~~285
Clinical Chemistry	4	3.5	14
Hematology	4	3	12
Serology	1	5	5
Total Volume of Blood Drawn			~~402~~449

3.5.2 Appropriateness of Measurements

The assessments that will be made in this study are standard and generally recognized as reliable, accurate, and relevant.

3.5.2.1 Timing of Assessments

For PK, predose samples will be obtained between waking up and dosing. Postdose samples up to 20 minutes postdose will be obtained with a time window of ±1 minute. Thereafter, postdose samples will be obtained with time margins of ±5% of the time that has passed since dosing. The ±5% time window also applies to the start and end times of urine collection intervals and to the total duration of each collection interval.

For safety assessments, predose assessments will be performed between waking up and dosing. For safety assessments up to 2.5 hours postdose, a time window of ±15 minutes is allowed. Thereafter, serial postdose assessments (eg, multiple assessments within any given day) will be performed with time margins of ±10% of the time that has passed since dosing.

When assessments are planned for the same scheme time, the order of the assessments should be arranged in such a way that PK blood sampling will be done after the ECG and vital signs recordings have been conducted, with PK blood sampling exactly on time.

3.5.3 Pharmacokinetic, Safety, and Exploratory Variables

3.5.3.1 Pharmacokinetic Variables

PK variables will be the plasma and urine concentrations of BTZ-043 and main metabolites, the total radioactivity concentrations in plasma, whole blood, urine, and feces (and in vomit, if produced) and their PK parameters. The PK parameters to be

determined or calculated using noncompartmental analysis include, but are not limited to, the parameters as given in Table 4. A complete list of PK parameters will be provided in the PK statistical analysis plan (SAP).

Table 4 Pharmacokinetic Parameters

Parameter	Description	Parent	Metabolites	TRA
C_{max}	Maximum observed plasma concentration	X	X	X
t_{max}	Time to attain maximum observed plasma concentration	X	X	X
AUC_{0-t}	Area under the plasma concentration-time curve up to time t, where t is the last point with concentrations above the LLOQ	X	X	X
AUC_{inf}	Area under the plasma concentration-time curve from time 0 to infinity calculated as: $AUC_{0-inf}=AUC_{0-t}+C_{last}/k_{el}$, where C_{last} is the last measurable plasma concentration	X	X	X
K_{el}	Terminal elimination rate constant	X	X	X
$t_{1/2}$	Terminal elimination half-life, calculated as $0.693/k_{el}$	X	X	X
CL/F	Apparent oral clearance, calculated as $dose/AUC_{0-inf}$	X		
V_z/F	Apparent volume of distribution at terminal phase	X		
CL_R	Renal clearance	X		
Ae_{urine}	Cumulative amount of drug excreted in urine	X	X	X
Ae_{feces}	Cumulative amount of drug excreted in feces			X
Ae_{vomit}	Cumulative amount of drug excreted in vomit, if produced			X
Ae_{total}	Total amount of drug excreted, calculated as $Ae_{total}=Ae_{urine}+Ae_{feces}(+Ae_{vomit})$			X
fe_{urine}	Fraction of the dose administered excreted in urine (%)	X	X	X
fe_{feces}	Fraction of the dose administered excreted in feces (%)			X
fe_{vomit}	Fraction of the dose administered excreted in vomit (%)			X
fe_{total}	Fraction of the dose administered excreted in urine, feces, and vomit (if produced) (%)			X

TRA=total radioactivity; LLOQ=lower limit of quantification

3.5.3.2 Safety Variables

The safety variables to be measured include, but are not limited to, the variables as given below. A complete list of safety variables will be provided in the SAP.

- AEs
- Clinical laboratory
- Vital signs
- 12-lead ECG
- Physical examination

3.5.3.3 Exploratory Variables

Metabolite profiling and metabolite identification will be conducted under a separate protocol/bioanalytical study plan, by Nuvisan Grafing, and the results will be reported separately. ~~The metabolite profiling/identification report will be included in the clinical study report (CSR) as an appendix, and results from the metabolite profiling/identification report will be briefly summarized in the CSR.~~

3.5.4 Drug Concentration Measurements

The analysis of BTZ-043 and main metabolites in plasma and urine samples will be performed at the Bioanalytical Department of Nuvisan Grafing using validated/qualified LC-MS. The analysis of whole blood, plasma, urine, and feces (and vomit, if produced) for total radioactivity will be conducted at the Bioanalytical Laboratory of PRA using a validated liquid scintillation counting method. The bioanalytical reports for the determinations will be included as appendices in the CSR.

The analysis of quick counts will be conducted at the Bioanalytical Laboratory of PRA using a validated method.

Metabolite profiling in plasma, urine, and feces samples by radiometry will be conducted at the PK Sciences & Biotransformation Department of Nuvisan Grafing. ~~The bioanalytical report for the metabolite profiling and identification will be included as appendix in the CSR.~~

3.5.5 Retention of Blood, Plasma, Urine, and Feces Samples

Blood and urine samples remaining after clinical laboratory assessments have been performed will not be stored for future use and will be destroyed after analysis as per laboratory procedure.

Blood, plasma, urine, and feces (and vomit, if applicable) samples remaining after total radioactivity measurements and after sample aliquotation for bioanalytical and metabolite identification have been performed, will be stored at the site of the Promotor and destroyed no later than 10 years after the date of the final CSR.

3.6 Statistical Procedures and Determination of Sample Size

3.6.1 Analysis Sets

3.6.1.1 Safety Set

All subjects who have received the single oral dose of ^{14}C-BTZ-043.

3.6.1.2 Pharmacokinetic Set

All subjects who have received the single oral dose of ^{14}C-BTZ-043 and provided sufficient bioanalytical assessment results to calculate reliable estimates of the PK parameters.

3.6.2 Statistical and Analytical Plan for Pharmacokinetic, Safety, and Exploratory Evaluation

A safety SAP will be generated by the Biostatistics Department of PRA and a PK SAP will be generated by Nuvisan; the SAPs will be finalized prior to database lock. Full details of the analysis to be performed will be included in the SAPs.

Any deviation from the SAPs will be reported in the section "Changes in Planned Analysis" in the CSR.

3.6.2.1 Pharmacokinetic Evaluation

The PK parameters and their statistical evaluation of BTZ-043 and *main* metabolites will be reported as ~~an integrated PK report, which will be included as an appendix and briefly summarized in the CSR.~~ *part of the bioanalytical report (included as appendix in the CSR). The PK parameters and their statistical evaluation of total radioactivity will be part of the mass balance report (included as an appendix in the CSR). A separate integrated PK report will incorporate the BTZ-043 and main metabolites' PK, the total radioactivity PK, and the metabolite profiling PK data for an integrated evaluation.*

All data will be summarized using descriptive statistics and will be listed and summarized in tabular and/or graphical form.

3.6.2.2 Evaluation of Safety and Tolerability

Safety and tolerability will be assessed through AEs, clinical laboratory, vital signs, ECGs, physical examination findings, and any other parameter that is relevant for safety assessment.

3.6.2.2.1 Adverse Events

A listing of all individual AEs will be provided. Summary tables of TEAEs will be presented by system organ class based on the MedDRA terminology list (preferred terms): 1 containing the number of TEAEs (frequency of occurrence, number of subjects experiencing the event) by treatment and 1 containing the number of drug-related TEAEs (frequency of occurrence, number of subjects experiencing the event) per treatment. Additional tables of total counts by treatment and relationship and by treatment and severity will be given.

3.6.2.2.2 Clinical Laboratory

Clinical laboratory data will be listed accompanied by an indication if the parameter is outside the reference range. A summary of all data outside the reference range of the clinical laboratory will be provided. Clinical laboratory data will be presented descriptively, where applicable.

3.6.2.2.3 Vital Signs and Electrocardiograms

Vital signs and ECG parameters will be listed and they will be presented descriptively, where applicable.

3.6.2.2.4 Physical Examination

Changes from baseline for physical examination will be described and listed.

3.6.2.3 Evaluation of Exploratory Variables

The PK parameters and their statistical evaluation of metabolites *not measured for the primary objectives* will be reported in ~~the~~ *a separate* integrated PK report.~~, which will be included as an appendix and briefly summarized in the CSR.~~

All data will be summarized using descriptive statistics and will be listed and summarized in tabular and/or graphical form.

The metabolite profiling and identification results will be reported separately (see Section 3.5.4).

3.6.3 Determination of Sample Size

Since the present study is a descriptive study aimed at assessing excretion routes of BTZ-043 by evaluating mass balance, a formal statistical sample size calculation was not performed. A sample size of 4 subjects is a minimum accepted number of subjects for AME studies and is considered sufficient to achieve the study objectives. Early-termination subjects may be replaced if the total number of completers drops below 4, after mutual agreement between the Sponsor and PRA.

3.7 Data Quality Assurance

The study may be audited by the Quality Assurance Department at PRA to assess adherence to the clinical study protocol (CSP) and Quality System. During the conduct of the study, process-related audits may be performed. An audit certificate will be provided in the appendices of the final CSR outlining any audits and other related activities performed.

The clinical research site will be monitored by the study monitor to ensure correct performance of the study procedures and assure that the study is conducted according to the relevant regulatory requirements. The eCRF entries will be verified with the source documentation, if applicable (in some cases data are captured directly on the eCRF, therefore source data verification is not necessary).

Regulatory authorities, the Independent Ethics Committee (IEC), and/or the Sponsor's clinical quality assurance group may request access to all source documents, eCRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

Quality control principles will be applied throughout the performance of this study. Review procedures will be followed at PRA for all documents that are generated in relation with the study.

An explanation will be given for all missing, unused, and spurious data in the relevant sections of the CSR.

4. ETHICS

4.1 Independent Ethics Committee

The submission package including the CSP and the ICFs will be submitted for review and approval by the IEC of the foundation "Beoordeling Ethiek Biomedisch Onderzoek" (English translation: "Evaluation of Ethics in Biomedical Research") (Dr. Nassaulaan 10, 9401 HK Assen, The Netherlands) prior to the eligibility screening. The composition of the IEC is in accordance with the recommendations of the World Health Organization, the International Council for Harmonisation (ICH) E6(R2) Guideline for Good Clinical Practice (GCP) (European Medicines Agency [EMA]/Committee for Medicinal Products for Human Use [CHMP]/ICH/135/1995)[4], and the EU Clinical Trial Directive (CTD) (Directive 2001/20/EC)[5] (see below). The submission package will also be shared with the CA in the Netherlands for a statement of no objection.

PRA will keep the IEC informed about the progress of the study. All changes in research activities and all unanticipated problems involving risks to human subjects will be immediately reported to the IEC. In accordance with Section 10, Subsection 1, of the Dutch law on Medical Research in Human Subjects (WMO, revised Dec 2015)[6], PRA will inform the subjects and the IEC if anything occurs on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal, or if further recruitment of subjects in the study has been put on hold for that reason, whichever occurs first. The study may be suspended pending further review by the IEC, except insofar as suspension would jeopardize the subjects' health. PRA will take care that all subjects are kept informed.

No changes will be made to the study without IEC approval, except when required to eliminate apparent immediate hazards to human subjects.

Notification of the end of the study will be sent by PRA to the CA in The Netherlands and to the IEC within 90 days after completion of follow-up for the last subject. In case a study is temporarily halted, PRA will notify the IEC immediately, including the reason for this. In case a study is ended prematurely, PRA will notify the IEC and the CA in The Netherlands within 15 days, including the reasons for the premature termination. A summary of the results of the study will be sent by PRA to the CA and the IEC within 1 year after the end of the study.

The written documentation of approval of the CSP and ICFs will be provided to the Sponsor before the start of the study. Any communication with the IEC and/or CA before, during, and/or after the study will be provided to the Sponsor.

4.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origin in the World Medical Association (WMA) Declaration of Helsinki, adopted by the 18th WMA General Assembly, Helsinki, Finland, Jun 1964, and subsequent amendments.[7]

This study is also designed to comply with ICH E6(R2) Guideline for GCP (EMA/CHMP/ICH/135/1995)[4], and the EU CTD Directive 2001/20/EC[5], as incorporated into Dutch Law.[6]

Guidelines adopted by the ICH and other relevant international guidelines, recommendations, and requirements will be taken into account as comprehensively as possible, as long as they do not violate local law.

Whenever the term "Investigator" is noted in the CSP text, it may refer to either the Investigator at the site or an appropriately qualified, trained, and delegated individual of the investigational site.

4.3 Subject Information and Consent

All subjects will be informed verbally and in writing regarding the objectives, procedures, and risks of study participation. The subjects will be given the time to ask questions. The subjects will have to sign the Dutch or English version of the ICF before any study-related procedures are started. The signed ICFs will be retained and archived at PRA and a copy will be provided to the subject. The ICF contains information about the objectives of the study, the procedures followed during the study, and the risks and restrictions of the study, with special reference to possible side effects of the study drug and potential interactions. In addition, insurance coverage provided during the study is explained. The elements addressed in the ICF are according to the ICH E6(R2) Guideline for GCP (EMA/CHMP/ICH/135/1995).[4]

4.4 Privacy

All personal details will be treated as confidential by the Investigator and staff at PRA, the Sponsor, and any sub-contractors involved, and handling of personal data will be in compliance with the EU General Data Protection Regulation.[8]

5. STUDY ADMINISTRATIVE STRUCTURE

5.1 Distribution of Activities

5.1.1 Preparation of Study Drug

The study drug will be prepared at the PRA Pharmacy.

5.1.2 Laboratory Assessments

The analysis of BTZ-043 and main metabolites in plasma and urine samples will be performed at the Bioanalytical Department of Nuvisan Grafing (Grafing, Germany) by using the Nuvisan study protocol G-A-LAB-21-004.

The analysis of total radioactivity in whole blood, plasma, urine, and feces (and vomit, if produced) will be performed at the Bioanalytical Laboratory of PRA (Assen, The Netherlands).

The analysis of clinical laboratory samples will be performed at the PRA Clinical Laboratory.

Metabolite profiling and identification will be performed at the PK Sciences and Biotransformation Department of Nuvisan Pharma Services GmbH (Grafing, Germany) by using the Nuvisan study protocol G-A-MET-21-002.

5.1.3 Electronic Case Report Form Design

The eCRF design will be performed with the computer program Oracle Clinical (Oracle, Redwood Shores, Redwood City, CA, US) by the Database Programming Department of PRA.

5.1.4 Data Management

Data management will be performed with the computer programs Oracle Clinical (Oracle, Redwood Shores, Redwood City, CA, US), SAS® (SAS Institute Inc, Cary, NC, US), and EXACT (Kinship EXACT™, Kinship Technologies, a technology subsidiary of PRA) by the Data Management Department of PRA.

5.1.5 Statistics

A safety SAP will be provided by the Biostatistics Department of PRA. The safety analysis will be conducted by the Biostatistics Department of PRA. Statistical analysis will be performed with the computer program SAS® (SAS Institute Inc, Cary, NC, US). A PK SAP will be provided by Nuvisan Grafing. Statistical evaluation of PK parameters will be conducted by Nuvisan Grafing. PK parameters will be calculated using Phoenix WinNonlin (Pharsight, Mountain View, CA, US). All individual results will be provided to the Sponsor after completion of the study.

5.1.6 Clinical Study Report Writing

The CSR, structured in accordance with the guideline "Structure and Content of Clinical Study Reports - ICH E3"[9], will be written by PRA and a draft version prepared for

approval by the Sponsor. The final version will be provided as paper version to the Sponsor.

5.2 Documentation

5.2.1 Archiving

All documents concerning the study will be kept on file in the Central Archives of PRA for at least 25 years after conduct of the study. The Sponsor will receive the completed eCRFs as PDF file.

5.2.2 Recording of Data in Source Documents and Electronic Case Report Forms

Wherever possible, all data will be entered directly into the eCRFs. Source documents will be used in some cases.

A data management plan will be written by the Data Management Department of PRA, which will be finalized prior to performing any data validation. An appendix to the data management plan (origin of source data list for data entry) will identify any data to be recorded directly in the eCRF (ie, no prior written or electronic record of data) and which data should be considered source data.

6. CONFIDENTIALITY AND PUBLICATION POLICY

All information generated in this study is considered highly confidential and must not be disclosed to any person or entity not directly involved with the study unless prior written consent is gained from the Sponsor and Promotor. However, authorized regulatory officials, the Sponsor and its authorized representatives are allowed full access to the records.

All study subjects must be informed that their personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject, who will be required to give consent for their data to be used as described in the ICF. The subjects must be informed that their medical records may be examined by auditors or other authorized personnel appointed by the Sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

Identification of subjects and eCRFs shall be by unique subject numbers only.

All personal details will be treated as confidential by the Investigator and staff at PRA.

All relevant aspects regarding publication will be part of the contract (or similar document) between the Sponsor and PRA.

7. REFERENCES

1. Investigator's Brochure BTZ-043. Edition 04. 10 Nov 2020.
2. CPMP/EWP/560/95/Rev. 1 Corr. 2** Committee for Human Medicinal Products (CHMP). Guideline on the investigation of drug interactions. 21 Jun 2012.
3. Radiation Burder Calculation Report EDS-NL. Dosimetry BTZ-043. 27 Nov 2018.
4. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E6(R2): Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice. Adopted by the European Medicines Agency (EMA), Committee for Human Medicinal Products, Document Reference EMA/CHMP/ICH/135/1995), 14 Jun 2017.
5. Directive 2001/20/EC of the European Parliament and of the Council of 4 Apr 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.
6. Medical Research Involving Human Subjects Act (WMO, Wet Medisch-Wetenschappelijk Onderzoek met Mensen), revision Dec 2015.
7. World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (18th WMA General Assembly 1964), revised at 64th WMA General Assembly, Fortaleza, Brazil, Oct 2013.
8. The General Data Protection Regulation (GDPR). Regulation (EU) 2016/679 of the European Parliament and the Council of the European Union, 27 Apr 2016, applicable as of 25 May 2018.
9. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E3: Structure and Content of Clinical Study Reports. Note for Guidance on Structure and Content of Clinical Study Reports, Adopted by the Committee for Human Medicinal Products, European Medicines Agency (EMA), Document Reference CPMP/ICH/137/95, Jul 1996.
10. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. Note for Guidance on Clinical Safety Data Management, Adopted by the Committee for Human Medicinal Products, European Medicines Agency (EMA), Document Reference CPMP/ICH/377/95, Jun 1995.

8. APPENDICES

8.1 Drug Accountability

Upon receipt of the study drug, it will be inspected and counted by the responsible pharmacist. If necessary, all study drug will be repacked per dosing occasion and labeled according to PRA SOPs.

The study drug will be kept in the PRA Pharmacy or in a locked and secured storage facility accessible to the pharmacist and the pharmacy assistant only. Temperature is monitored 24/7 with an automatic environmental monitoring system. In case of an alarm, the pharmacist or pharmacist on duty is informed immediately by email and phone.

The responsible pharmacist will keep an inventory. This will include a description of the formulation and the quantity of study drug received for the study and a record of what is dispensed, to whom, and when.

On termination of the study, the responsible pharmacist will conduct a final inventory of the study drug supply and will record the results of this inventory in the Drug Accountability Form. Unused study drug will be returned to the Promotor upon notice is given to Promotor and Sponsor or will be locally destroyed according to PRA standard procedures. Final accountability needs to be verified by monitor before return and/or destruction of medication.

8.2 Adverse Events and Serious Adverse Events Evaluation and Reporting

8.2.1 Adverse Events

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. AE definitions will be followed as stated in the “Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting” (ICH topic E2A).[10]

All AEs reported by the subjects or apparent from their physical appearance during the clinical study will be reported on the AE eCRF page.

The severity of AEs will be graded using the most current version of the MedDRA:

- **Mild**: An AE that is easily tolerated by the subject, causes minimal discomfort, does not interfere with everyday activities, and does not require intervention.
- **Moderate**: An AE that is sufficiently discomforting to interfere with normal everyday activities; intervention may be needed.
- **Severe**: An AE that prevents normal everyday activities; treatment or other intervention usually needed.

If an AE has multiple aspects, the aspect with the highest severity will be graded.

It is emphasized that the term severe is a measure of severity; thus, a severe AE is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

In addition, clinically significant changes in physical examination findings and abnormal objective test findings (eg, laboratory, x-ray, ECG) should also be recorded as AEs. Test findings and physical examination findings can result in AEs if they:

- Are associated with accompanying symptoms, and/or
- Require additional diagnostic testing or medical/surgical intervention, and/or
- Lead to a change in study dosing or discontinuation from the study; result in the addition of significant additional concomitant drug treatment or other therapy, and/or
- Lead to any of the outcomes included in the definition of an SAE, and/or
- Are considered to be an AE by the Investigator or Sponsor.

Reporting as an AE should not be triggered by:

- Merely repeating an abnormal test, or
- Any abnormal test result that is determined to be an error.

The relationship of any AE to the study drug will be assessed and graded as related or not related.

Related:

- The AE follows a reasonable temporal sequence to study drug administration, and cannot be reasonably explained by the subject's clinical state or other factors (eg, disease under study, concurrent diseases, or concomitant medications).
- The AE follows a reasonable temporal sequence to study drug administration, and is a known reaction to the drug under study or a related chemical group, or is predicted by known pharmacology.

Not Related:

The AE does not follow a reasonable sequence from study drug administration, or can be reasonably explained by the subject's clinical state or other factors (eg, disease under study, concurrent diseases, and concomitant medications).

8.2.2 Serious Adverse Events

An SAE is any untoward medical occurrence that, on the basis of medical and scientific judgment:

- Results in death, or
- Is life-threatening (this refers to an event in which the patient 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), or
- Requires inpatient hospitalization for a medical reason or prolongation of existing hospitalization (this refers to hospital admission required for treatment of the AE). (Note: this does not include confinement in, for example, a respite unit; a skilled

nursing unit; rehabilitation facility; the clinical research center; or confinement due to planned or unplanned reason unrelated to study), or

- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Medical and scientific judgment should be exercised in deciding whether expedited 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 patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization.

SAEs will be collected from admission until the final study visit. SAEs that are related to the investigational drug and continue beyond the normal collection period (ie, are ongoing at the time a subject exits the study) will be followed until resolution or until stabilized with sequelae. SAEs that begin after the subject's participation in the study is complete, but that the Investigator considers to be related to study drug, may be reported at any time.

The Investigator or clinical site personnel must notify the Sponsor's Medical Monitor and PRA Drug Safety Center of all SAEs, regardless of relationship to the investigational drug, within 24 hours of clinical site personnel becoming aware of the event. The Investigator will provide the initial notification by sending a completed "SAE Notification Form," which must include the Investigator's assessment of the relationship of the event to investigational drug and must be signed by the Investigator.

In addition, notification is sent by PRA to the IEC and the subject's General Practitioner.

Follow-up information, or new information regarding an ongoing SAE, must be provided promptly to the Sponsor's Medical Monitor and PRA Drug Safety Center.
All SAE reports should be sent to the contacts provided on Page 4: SAE Contact Information.

8.2.3 Suspected Unexpected Serious Adverse Reactions

An SAE that is also an unexpected adverse drug reaction is called a suspected unexpected serious adverse reaction (SUSAR). Unexpected adverse reactions are adverse reactions of which the nature or severity is not consistent with the applicable product information (eg, investigator's brochure for an unapproved investigational medicinal product or the Summary of Product Characteristics for an authorized medicinal product).

The Sponsor or its representative (eg, PRA if agreed to before start of the study) will promptly report (expedited reporting) the following SUSARs to the IEC:

- SUSARs that have arisen in the current clinical study that was assessed by the IEC

- SUSARs that have arisen in other clinical studies of the same Sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the current clinical study that was assessed by the IEC

The Sponsor will promptly report (expedited reporting) all SUSARs to the CA and the Medicine Evaluation Board (MEB) of the country where this study is conducted and to the CAs in other Member States, as applicable.

SUSARs that have already been reported to the EMA Eudravigilance database do not have to be reported again to the CA and the MEB because they have direct access to the Eudravigilance database.

Expedited reporting will occur no later than 15 calendar days after the Sponsor or its representative has first knowledge of the adverse reactions. For fatal or life-threatening cases, the term will be maximally 7 calendar days for a preliminary report with another 8 days for completion of the report.

8.2.4 Follow-up of Adverse Events

Follow-up of AEs will continue until resolution, stabilization, or death. In case of ongoing AEs at database closure, the data obtained at database closure will be used in the statistical analysis. The follow-up of the AE will be documented in the source documents and will be described in the final CSR only if considered relevant by the Investigator.

8.3 Pregnancy

If the Investigator becomes aware of a pregnancy occurring in the partner of a male subject participating in the study up to 90 days after the last dose of study drug of the male subject, the pregnancy should be reported to the Sponsor within 1 working day of obtaining written consent from the pregnant partner. The Investigator may make arrangements for the partner to be counseled by a specialist, to discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the partner should continue untile the outcome of the pregnancy is known.