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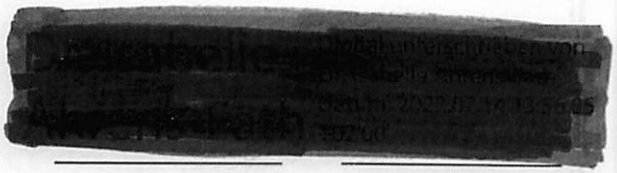
1 SIGNATURE PAGE FOR BENFOVIR AG

Hereinafter called benfovir AG

Investigational drug name: benfo-oxythiamine (B-OT)

Study number: BV-01-101



Dr. Isabelle Ahrens-Fath,
CSO/CDO,
benfovir AG

Signature Date

2 SIGNATURE PAGE FOR INVESTIGATOR

Investigational drug name: benfo-oxythiamine (B-OT)

Study number: BV-01-101

I agree to the terms and conditions relating to this study as defined in this Protocol, electronic Case Report Form (eCRF), and any other protocol-related documents. I fully understand that any changes instituted by the investigator(s) without previous agreement with the sponsor would constitute a violation of the protocol, including any ancillary studies or procedures performed on study subjects (other than those procedures necessary for the well-being of the subjects).

I agree to conduct this study in accordance with the Declaration of Helsinki and its amendments, International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines and applicable regulations and laws. In particular, I will obtain approval by an Ethics Committee (EC) prior to study start and signed informed consent from all subjects included in this study. In addition, I will allow direct access to source documents and agree to inspection by auditors from the sponsor and Health Authorities. I will ensure that the study drug(s) supplied by the sponsor are being used only as described in this protocol. Furthermore, I confirm herewith that the sponsor is allowed to enter and utilize my professional contact details and function in an electronic database for internal purposes and for submission to worldwide Health Authorities.

[redacted] [redacted]

Principal Investigator Signature Date

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4 LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	alanine aminotransferase/serum glutamic pyruvic transaminase (SGPT)
ANOVA	Analysis of variance
AST/SGOT	Aspartate aminotransferase
ATD	Anticipated Therapeutic Dose Range
AUC	Area Under the Curve
BASO	Basophil
B-OT	Benfo-oxythiamine
BMI	Body Mass Index
BP	Blood Pressure
Bpm	beats per minute
BUN	Blood Urea Nitrogen
CA	Competent authority
Cl/F	Clearance
C_{max}	Maximum concentration
EC	Ethics Committee
eCRF	electronic Case Report Form
EOS	Eosinophil
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
DCC	Diagnostics and Consultation Centre
DMP	Data Management Plan
EC	Ethics Committee
ECG	Electrocardiogram
EDTA	Ethylene Diamine Tetra-acetic Acid
EOS	End of study
EU	European Union
FIH	First in human
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
Gamma-GT	Gamma-glutamyltransferase
HCT	Hematocrit
HGB	Hemoglobin
Hem	Hematology
Hep B	Hepatitis B
Hep C	Hepatitis C
HIV	Human Immunodeficiency Virus

HR	Heart Rate
hr.	Hour
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
kg	Kilogram
LLOQ	Lower Limit of Quantification
LYMP	Lymphocyte
MAD	Multiple Ascending Dose
MedDRA	Medical Dictionary for Regulatory Activities
MCV	Mean Corpuscular volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MONO	Monocyte
NEUT	Neutrophil
NOAEL	No Observed Adverse Effect Level
OTC	Over-the-counter
OT	Oxythiamine
PAD	Pharmacologically Active Dose
PD	Pharmacodynamic
PE	Physical examination
PI	Principal Investigator
PK	Pharmacokinetic
PLT	Platelet
PPP	Pentose phosphate pathway
PR	Pulse Rate
PT	Preferred Term
RBC	Red Cell Count
R5P	Ribose-5-Phosphate
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SAS	Statistical Analysis Software
SOC	System Organ Class
SOP	Standard Operating Procedure
SRC	Safety Review Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
TK	Toxicokinetic

TKT	Transketolase
TKTL1	Transketolase-like 1
$t_{1/2}$	Terminal half life
t_{max}	Time of the maximum concentration
UADR	Unexpected Adverse Drug Reaction
UAE	Unexpected Adverse Event
ULOQ	Upper limit of quantification
WBC	White Cell Count
WHO	World Health Organization

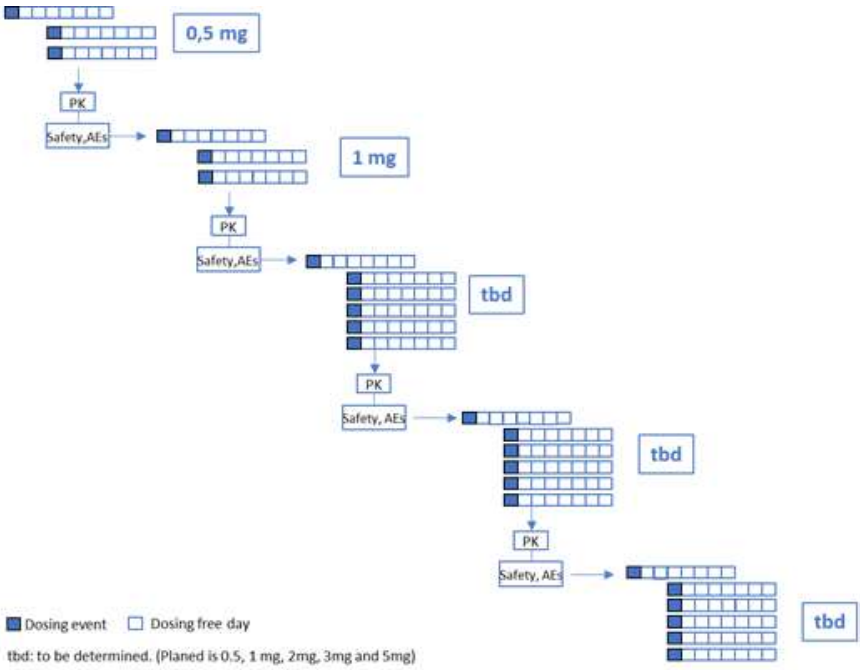
5 PROTOCOL SYNOPSIS

Study Title	Assessing the safety, tolerability and pharmacokinetics of Benfo-Oxythiamine (B-OT) in healthy volunteers – An Open label, phase I Study
Study Type	Monocentric, open label, first-in-man phase I study
Protocol No. / Short Title	BV-01-101 / BOOST
EUDRA-CT No	2021-005616-60
Targeted Indication	SARS-CoV-2 infection / Covid-19
Investigational Drug	Benfo-oxythiamine (B-OT) B-OT is a novel thiamine analogue, which functions as a prodrug and releases oxythiamine in the human body. The introduction of a benfo group into a molecule improves its cell permeability and may ensure better bioavailability. The thiamine antagonist oxythiamine is an irreversible inhibitor of thiamine-dependent enzymes, such as transketolases, and efficiently inhibits the sugar metabolism and the accumulation of Ribose-5-Phosphate (R5P) building blocks.
Sponsor	benfovir AG Gräfenhäuserstr. 26, 64293 Darmstadt Germany

Objectives and end point	Primary Objectives: To assess the safety and tolerability of single and multiple ascending doses of B-OT • Endpoints: o safety evaluations including safety laboratory assessments, physical examination (PE), electrocardiograms (ECGs), vital signs, adverse events (AEs) and serious adverse events (SAEs). Secondary Objectives • To evaluate the pharmacokinetics (PK) of benfo-oxythiamine (B-OT) and its active metabolite oxythiamine (OT) in plasma following single doses administered to healthy volunteers • To evaluate the pharmacokinetics (PK) of benfo-oxythiamine (B-OT) active metabolite oxythiamine (OT) in plasma following multiple doses administered to healthy volunteers. • Endpoints: o SAD: Plasma PK parameters, including Cmax, tmax, t1/2, CL/F, λz, Vz/F, AUC0-inf and AUC last as appropriate o MAD: The following PK endpoints will be derived from plasma oxythiamine (OT) concentration versus time data following administration according to the dosing schedule on Day 1: AUCτ, Cmax, tmax, on Day 7: AUCτ, Cmax,ss, tmax,ss, Swing, PTF%, CLss/F, Vz/F, Ctrough, Cav, Cmin, t1/2, AUCinf and AUClast as appropriate and Day7/Day1: Ra(AUCτ), Ra(Cmax). Exploratory Objectives • To evaluate the pharmacodynamics (PD) by measuring the transketolase activity in • To analyse PK parameters of other B-OT derivative metabolite(s), if any.

Study Design, **Mode of administration, starting dose and dose level,** **Stopping rule**	This is a monocentric, open-label, non-randomized, uncontrolled, first-in-human study to assess the safety, tolerability, and pharmacokinetics /pharmacodynamics of B-OT in healthy subjects. This study has two parts: a single ascending dose (SAD) part and a multiple ascending dose (MAD) part. **Administration and dose levels:** **First Part of the study: SAD** In the SAD part, a single dose of B-OT will be administered per oral (*p.o.*) once daily on day 1 followed by 7 days without dosing. Up to 5 cohorts will be enrolled and dosed. Dosing is based on NOAEL x HED x 1/10 (No Observed Adverse Effect Level x Human Equivalent Dose [dog/human] x Safety Factor). As NOAEL was established in dog after a 7-day treatment and B-OT has already been used in compassionate use at a minimum daily dose of 3 mg for 7 days in 5 Covid-19 patients and 5 late-stage prostate cancer patients without any safety issue, only 3 subjects will receive the two first lowest doses. The first dose of B-OT will be 0.5 mg (0.0083 mg/kg body weight, average 60 kg adult). After the first cohort, enrolment will be halted. If no safety issue is observed, the second dose level will be 1 mg (0.016 mg/kg body weight). The first and second dose level cohort will have 3 subjects. The human PK/PD model will be refined before enrolling to the next dose level. As this is the first study testing B-OT in humans, study design adjustments may be made based on emerging data from each dose cohort based on review of preliminary safety, tolerability, and PK/PD results. If these data allow for, the dose escalation scheme of 2 mg, 3 mg and 5 mg will follow in 3 additional cohorts of 6 subjects. Each cohort will start with a sentinel subject (subject #1). Subject #2 - #3 or #2 - #6 of the same cohort will start treatment after a minimum of 48 hours if safety data indicate that dosing of subsequent subjects of this cohort is justified.

The following figure represents the study design of the SAD part:



The filled box represents a dosing event, a white box a represents a dosing free day. Single dose treatment will be administered on Day 1.

Dose escalation to the next dose level will occur if three (first and second cohort) or six subjects (3 next cohorts) have completed the single dose treatment and if PK as well as safety data allow an escalation to the next dose level.

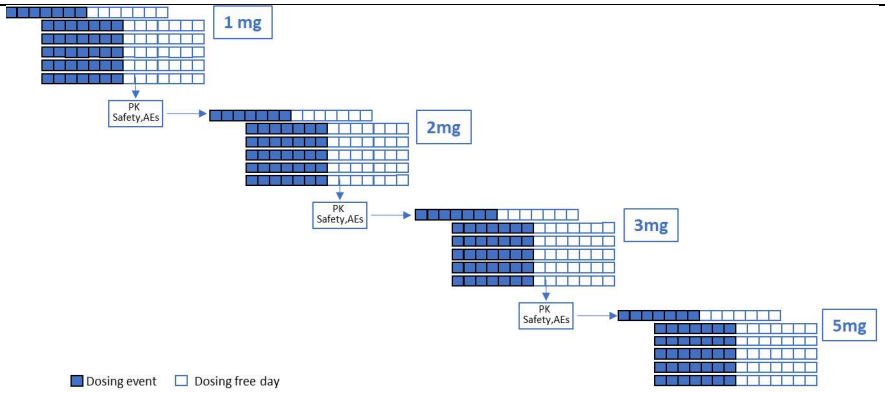
Second Part of the study: MAD

In the MAD part, a single dose of B-OT will be administered orally (*p.o.*) once daily for 7 days (D1-D7) followed by 7 days without dosing. The first dose will be 1 mg, as the PK and safety data in the SAD part of the study have shown an acceptable safety margin at this dose (see IB).

Each cohort will start with a sentinel subject (subject #1).

Subjects #2 - #6 of the same cohort will start treatment after a minimum of 72 hours if no adverse event is observed in the sentinel subject.

The following figure represents the study design of the MAD part:

	 **Definite Stopping rules** Dose escalation will not continue to the next dose level if (a) maximal OT plasma levels are achieved, i.e. further increase is not justified from a scientific point of view, or if (b) toxicities are observed that do not justify continuation from an ethical point of view. The study will be halted if one or more subjects experience a serious adverse event (SAE) that is considered as related to B-OT or 2 or more subjects in the same group experience severe AEs that are considered to be related to B-OT. Dose-escalation will stop if clinically relevant signs or symptoms of similar nature occur in 2 or more subjects in a group that, in the opinion of the PI, warrant stopping of dose-escalation.
Inclusion criteria	Subjects will be enrolled if they meet all the **following inclusion criteria:** - Signed and dated informed consent form - Subjects capable to understand the purposes and risks of the study, and who are willing and able to participate in the study - Male volunteers who are willing to comply with contraception requirements - Aged 18-60 years, inclusive - Healthy participants, as determined by screening assessments and Principal Investigator's judgment - Healthy status is defined by the absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECG, hematology, blood chemistry, serology, and urinalysis - Body Mass Index (BMI) of 18-30 kg/m^2 inclusive Contraception Requirements

	- Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame: - Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent - Use a male condom plus partner use of a contraceptive method with a failure rate of < 1% per year when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant. Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.
Exclusion criteria	- Subjects who meet any of the following **exclusion criteria** at Screening (unless otherwise stated) will not be eligible for participation: - Subjects who have a clinically relevant history as determined by the Investigator, or presence of respiratory, gastrointestinal, renal, hepatic, hematological, lymphatic, neurological, cardiovascular, psychiatric, musculoskeletal, genitourinary, immunological, dermatological, endocrine, connective tissue diseases, or disorders. - Fridericia's correction factor for QT (QTcF) > 450 ms or history of QT interval prolongation. - Have acute gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, heartburn) at the time of screening or admission. - Has any abnormal laboratory value of Grade 2 or higher, which is considered as clinically significant by the PI (or designee). - Has values of ≥ 10 % above the upper limit of normal for the following laboratory analytes: - alanine aminotransferase (ALT/SGPT), alkaline phosphatase (serum), aspartate aminotransferase (AST/SGOT), at Screening and Day-1 - creatinine and urea concentration in blood at Screening and Day-1 - Have a clinically relevant surgical history, as determined by the Investigator. - Have a history of relevant drug hypersensitivity. - Have a history of alcoholism or drug abuse. - Have a significant infection or known inflammatory process at screening or admission.

	- Have used any prescription or non-prescription medicines within 2 weeks of admission, unless in the investigator's opinion it will not affect the determination of safety or other study assessments. - Have received any investigational drug within 30 days prior to screening. - Have used tobacco or nicotine products within 3 months of screening - Has a positive alcohol or drug screen at Screening or the Day1 visit - Have donated or received any blood or blood products within the 3 months prior to screening. - Cannot communicate reliably with the investigator. - Have been vaccinated with a Covid-19 vaccine within 2 weeks prior to screening and/or during participation in the clinical trial - Are unlikely to co-operate with the requirements of the study.
Statistical considerations and sample size	The study is exploratory and is not powered to address any pre-defined hypotheses. The assessment of safety and tolerability will be performed on the safety analysis set, which includes all subjects who received at least one dose of B-OT. Complete description of all statistical analyses and methods will be presented in the Statistical Analysis Plan (SAP). The SAP will be reviewed and approved by the Sponsor and will be finalized prior to database lock. Plans for PK analyses will be included in the SAP. **Determination of Sample Size:** The sample sizes for the SAD and MAD cohorts are typical for a Phase 1 first in human (FIH) study. The total number of subjects accrued in the study will depend on the number of dose levels, but is currently set to a maximum of 48 subjects (in the SAD: 2 cohorts with 3 subjects and 3 cohorts with 6 subjects each and in the MAD: 4 cohorts of 6 subjects each). The safety and tolerability of B-OT will be analyzed by appropriate descriptive statistics. All other secondary endpoints will be summarized descriptively. **Study Populations:** Safety Population: All subjects who receive at least one dose of study drug. Pharmacokinetic Population: All subjects receiving study drug who comply sufficiently with protocol requirements (i.e., no major protocol deviations) and for whom enough analyzable PK samples have been obtained to allow the determination of the PK profile for B-OT/OT and the statistical analysis of the results.

	Safety Analyses: Statistical methods for the safety analyses will be descriptive in nature. Safety data, including AEs, clinical laboratory data, vital signs, ECG parameters, and PEs. All appropriate AEs will be graded using the DMID toxicity scale (March 2014). Change from baseline will be included in summary tables for laboratory parameters. All laboratory data will be included in the data listings and all test values outside the normal range will be flagged. **Pharmacokinetic Analyses:** Plasma PK parameters for each dose level in the SAD part of the study will be calculated from the concentrations of B-OT and OT measured in pre-dose and post-dose plasma samples. For each dose level, descriptive statistics will be presented. Figures will be created to display mean and individual subject B-OT and OT concentration versus time. Plasma PK parameters for each dose level in the MAD part of the study will be calculated from the concentrations of OT and possible derivate metabolites. Figures will be created to display mean and individual subject OT concentration versus time. **Interim Analyses:** No formal interim analyses are planned for this study. Data from SAD cohorts were frozen and analyzed prior to conducting the second MAD part of the study.

TABLE 1: VISIT AND ASSESSMENT SCHEDULE OF THE SINGLE ASCENDING DOSE PART

Visit	**Screening (Days -28 to -2)**	**Day -1**	**Day 1 (T=0)**	**Day 2 (24 hr)** +/- 30min	**Day 3 (48 hr)** +/-1hr	**Day 4 (72 hr)** +/-1hr	**Day 8 (EOS) (168 hr)** +/-2hr	
Informed consent	x							
Demography	x							
I/E; Medical history	x	x						
Physical examination-PE[1]	x	x	x[2]	x		x	x	[1]: a complete PE will be performed at Screening and/or Check-in and EOS; on all other days, a limited PE will target any change from the previous status, or any body system deemed pertinent to that subject by the PI (or designee) [2]: at 4 hr post-dose
Vital signs[3]	x	x	x	x	x	x	x	[3]: vital signs include pulse rate, respiratory rate, blood pressure and body temperature; on Day1, VS should be taken pre-dose and at 2,4, 8 and 12 hr post-dose
Laboratory assessment (hematology, biochemistry)	x	x[4]		x		x	x	[4]: Results for safety blood samples must be available and reviewed before drug is administered
Urine analysis	x	x		x	x	x	x	
ECG	x		x[5]	x			x	[5]: the baseline ECG should be conducted prior to dosing on day 1
SARS CoV antigen	x						x	
SARS CoV PCR		x				x		

Visit	**Screening (Days -28 to -2)**	**Day -1**	**Day 1 (T=0)**	**Day 2 (24 hr)** +/-30min	**Day 3 (48 hr)** +/-1hr	**Day 4 (72 hr)** +/-1hr	**Day 8 (EOS) (168 hr)** +/-2hr	
Viral screening (HIV, Hepatitis)	x							
Drug screening	x	x					x	
Breath alcohol test	x	x					x	
Weight/height (BMI)	x	x[6]					x[6]	[6]: only weight on Day -1 and 8
Confinement		x	x	x	x	x		
Ambulatory visit	x						x	
Administer study drug			x[7]					[7]: T=0 is time of drug administration
Plasma PK sample collection			x[8]	x[9]	x[9]	x[9]	x[9]	[8]: PK samples should be collected pre-dose and at 0.25, 0.5,0.75,1,1.5,2,4,8,12 hr. depending on ongoing PK data review, timepoints may be modified or removed. [9]: PK samples should be collected at 24,36,48, 72 and 168 hr post-dose on Day 2,3, 4 and 8
PD (TKT actvity)			x[10]	x	x	x	x	[10]: on Day 1, TKT blood sample should be taken pre-dose and at 1,2,4,8, and 12 hr and then at 24, 48, 72 and 168 hr post-dose.
Adverse Events	x[11]	x[11]	x[11]	x	x	x	x	[11]: Adverse events occurring prior to study drug administration should be recorded as medical history unless the event is directly related to study specific procedure
Prior and/or Concomitant medications	x	x	x	x	x	x	x	

TABLE 2: VISIT AND ASSESSMENT SCHEDULE OF THE MULTIPLE ASCENDING DOSE PART

Visit	Screening (Days -28 to -2)	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 11	Day 14 (EOS)	
Informed consent	x												
Demography	x												
I/E; Medical history	x	x											
Ambulatory visit	x										x	x	
Confinement		x	x	x	x	x	x	x	x	x			
Administer study drug			x	x	x	x	x	x	x				
Physical examination-PE[1]	x	x	x	x	x	x	x	x	x	x	x	x	[1]: a complete PE will be performed at Screening and/or Check-in and EOS; on all other days, a limited PE will target any change from the previous status, or any body system deemed pertinent to that subject by the PI (or designee)
Vital signs[2]	x	x	x	x[3]	x	x	x[3]	x	x[3]	x	x	x	[2]: vital signs include pulse rate, respiratory rate, blood pressure and body temperature; if not otherwise indicated, VS should be taken either at pre-dose on dosing days or at time of visit on ambulatory visit days [3]: on Day 2, 5 and 7, VS should be taken pre-dose and at 4 hr post-dose
Laboratory assessment (hematology, biochemistry, coagulation)	x	x[4]		x[4]			x[4]			x	x	x	[4]: Results for safety blood samples must be available and reviewed before drug is administered on Day 1, 3 and 6
Urine analysis	x	x		x			x			x	x	x	
ECG	x		x[5]	x		x			x			x	[5]: the baseline ECG should be conducted prior to dosing on Day 1, and subsequent ECGs should be taken at pre-dose on the rest of the dosing days and at time of visit on ambulatory visit days
SARS CoV antigen	x	x										x	

Visit	Screening (Days -28 to -2)	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 11	Day 14 (EOS)	
Viral screening (HIV, Hepatitis)	x												
Drug screening	x	x										x	
Breath alcohol test	x	x										x	
Weight/height (BMI)	x	x[6]				x[6]				x[6]	x[6]	x[6]	[6]: only weight
Plasma PK sample collection			x[7]	x[8]	x[8]	x[8]	x[8]	x[8]	x[7]	x[8]	x[8]	x[8]	[7]: PK samples should be collected pre-dose and at 0.25, 0.5,0.75,1,1.5,2,4,8,12 hr, on Day 1 and Day 7. Depending on ongoing PK data review, timepoints may be modified or removed. [8]: PK samples should be collected pre-dose on Day 2, 3, 4,5 and 6 and 24hr +/- 30 min (Day 8), 96hr +/- 4hr (Day 11) and 168hr +/- 4hr (Day 14) after last dosing of Day 7.
PD[9]			x[9]	x		x[9]	x		x[9]	x			[9]: PD blood sample should be taken pre-dose and at 1,2,4,8, and 12 hr after dosing on Day 1, Day 4 and Day 7. One sample per day should be taken on Day 2 and Day 5 pre-dose, corresponding to 24 h after previous day dosing. One sample should be taken 24 h after previous day dosing on Day 8.
Adverse Events	x[10]	x[10]	x[10]	x	x	x	x	x	x	x	x	x	[10]: Adverse events occurring prior to study drug administration should be recorded as medical history unless the event is directly related to study specific procedure
Prior and/or Concomitant medications	x	x	x	x	x	x	x	x	x	x	x	x	

6 INTRODUCTION AND RATIONALE

6.1 Introduction

Benfo-oxythiamine (B-OT) is a novel inhibitory thiamine analogue that acts as a prodrug and releases oxythiamine in the human body. B-OT is a previously unknown dosage form of oxythiamine which, like the drug benfotiamine (trade name Milgamma), has a lipophilic side group, the benfo group. Its introduction into a molecule improves its cell permeability and can thus ensure better bioavailability [Bitsch et al.,1991].

The active substance in the investigational medicinal product capsules is the small molecule benfo-oxythiamine (B-OT). B-OT represents a novel inhibitory thiamine (vitamin B1) analog and prodrug which is converted in the human body into oxythiamine. Oxythiamine is a thiamine antagonist and inhibits transketolase, the enzyme that controls the nonoxidative branch of the pentose phosphate pathway (PPP). Via the intermediate ribose-5-phosphate, the PPP supplies ribonucleotides for SARS CoV 2 replication. Therefore, it is hypothesized that the inhibition of transketolase by B-OT will reduce the capacity for viral replication.

SARS-CoV-2 (severe acute respiratory syndrome coronavirus type 2) is a new coronavirus that was identified as the cause of COVID-19 in early 2020. In March 2020, the WHO declared the outbreak of SARS-CoV-2 infections a pandemic.

The course of a SARS-CoV-2-infection varies in symptoms and severity, from asymptomatic infections to severe pneumonia with lung failure, multiple organ failure and death. The most common symptoms include cough, fever, rhinitis, sore throat, gastrointestinal symptoms and neurologically associated symptoms such as the loss of the sense of smell or taste [Lechien JR, et al. 2019; Yan CH, et al. 2020; Tong Jy, et al. 2020]. Other neurologically associated symptoms include headache, dizziness, cognitive impairment, and fatigue, which can also occur after mild courses and can manifest long after the acute SARS-CoV-2 infection has already resolved [Schenk M 2020; Li YC, et al. 2020; Whittaker A, et al. 2020; Kennedy M, et al. 2020; Hampshire A, et al. 2020]. This can significantly compromise the long-term health and quality of life of patients.

Some SARS-CoV-2 patients develop COVID-19 disease, which is associated with lung damage, oxygen deficiency and respiratory distress. Pneumonia may develop, usually in the second week of illness, and may progress to ARDS (Acute Respiratory Distress Syndrome), requiring ventilation and intensive care and possibly oxygenation of the blood outside the body (ECMO) [Yang X, et al. 2020; Graselli G, et al. 2020; Rieg S, et al. 2020]. Moreover, COVID-19 can also manifest in other organ systems. The sites of manifestation depend, among others, on the density of ACE-2 receptors (entry portal of the virus into the cell) in the tissues. High ACE-2 density exists in the respiratory tract, as well as in the intestine, vascular cells, kidney, heart muscle and other organs.

Severe courses of SARS-CoV-2 infection are often associated with an excessive reaction of the immune system („cytokine storm“). The virus can invade lung epithelial cells and alveolar macrophages and induce production of viral nucleic acid by use of cellular enzymes. The virus infection causes a metabolic switch from mitochondrial energy release (OxPhos) to a glucose-based energy release (aerobic glycolysis). This metabolic switch stimulates the release of large amounts of cytokines, as well as activates macrophages, dendritic cells, and others, leading to multisystem organ failure and death. Inhibition of glucose metabolism by 2-deoxy-glucose (2DG) inhibits virus replication in infected cells confirming the critical role of glucose metabolism.

Drugs that inhibit immune system activity, such as dexamethasone, show positive effects on severe disease courses. Nevertheless, a high proportion of patients undergoing a severe course of SARS-CoV-2 infection die. According to the study of critical illness courses during the first COVID-19 epidemic, 47% of cases treated with intensive care died [Schilling J, et al. 2020].

Containment of the SARS-CoV-2 pandemic is massively complicated by the fact that, firstly, SARS-CoV-2 infections can also proceed asymptomatic, and therefore apparently healthy people infect others, thus facilitating the further expansion of the virus. Secondly, new and faster-spreading viral mutations have now emerged and can lead to a sudden increase in the number of cases. This poses the risk of overloading the healthcare system and hospitals – as is currently the case, for example, in the UK, Portugal and Brazil.

B-OT is a novel drug candidate in development as a therapy for COVID-19. It is intended that patients diagnosed with COVID-19, i.e., people with disease symptoms and confirmed diagnosis of SARS-CoV-2 viral infection, will be treated with B-OT capsules. It is anticipated that B OT will actively interfere with viral replication and lead to fast recovery and cure of COVID 19. In consequence, the risk for disease aggravation and hospitalization will be reduced, as well as long-term fatigue and similar lingering symptoms experienced by some COVID-19 patients.

The clinical development program will investigate the potential of B-OT to shorten the time to recovery from COVID-19 and to prevent hospitalization. It will further generate data on safety and potential adverse reactions.

In the FIH clinical trial, safety will be assessed through the incidence and severity of adverse events at each of the dose levels tested. Furthermore, for clinical and laboratory parameters change from baseline will be assessed, to assess the overall safety profile and tolerability of B-OT.

In subsequent clinical investigations – to be conducted in patients with SARS-CoV-2 infection and COVID-19 – the above evaluations will be complemented with clinical efficacy assessments, such as, but not limited to, overall response, prevention of progression, and reduction in the need for oxygen supplementation.

To support the clinical development of B-OT, several non-clinical safety studies have been performed using the oral route of administration, according to the requirements of the M3(R2) ICH guideline (Nonclinical safety studies for the conduct of human clinical trials) [M3 (R2) ICH Guideline S9, EMA 2009]. All pivotal toxicology and toxicokinetic (TK) studies reported in this Investigator's Brochure (IB) were conducted in accordance with the Good Laboratory Practice (GLP) principles. Pilot, exploratory, and ADME studies were not conducted under strict GLP procedures but were conducted using appropriate protocols and documentation to ensure data integrity. In the in vivo studies as performed, B-OT was administered as a solution in drinking water, by oral (gavage) administration.

The clinical development of B-OT has started. The single ascending dose (SAD) part of this study assessing the safety, tolerability, and pharmacokinetics of B-OT in healthy volunteers has been completed and the multiple ascending part (MAD) will start as soon as the amendment of this protocol has been approved.

The SAD part of the study showed good tolerability and there were no side effects detected after once-daily administration of B-OT at an escalating dose ranging from 0.5 mg to 5 mg.

No B-OT was detected at any dose level in the plasma of the healthy volunteers.

At the maximum dose of 5 mg, the maximum concentration of OT in human plasma was 15.45± 3.76 ng/ml. The time to reach the maximum concentration ranged at the different dose between 1.67±0,29 and 2.50±1,18 hours.
Following single administrations of B-OT, the exposure to OT in terms of Cmax and AUC0-t increased with ascending doses from 0.5 to 5 mg in a slightly over proportional manner.
Further details can be found in the IB.

Study Rationale

This is a FIH clinical study and as such is conducted primarily to obtain information on safety, PK and PD properties of the tested compound. The scientific rationale of the study is based on the information that RNA viruses such as SARS-CoV-2 bring their own RNA polymerase into the cell. However, after infecting the cell and releasing their viral RNA, they depend on precursors necessary for replication of their viral RNA. The virus is dependent on ribose-5-phosphate (R5P) from the host cell as an essential building block for their replication.

The central switch for the regulation of cell cycle and cell proliferation as well as for the sufficient supply of R5P building blocks is provided by transketolase-like 1 (TKTL1) [Li et al., 2019], which therefore represents a good target for antiviral therapy.

Transketolases (TKT) are key enzymes of the pentose-phosphate-pathway (PPP). They form dimers and are activated by the binding of thiamine diphosphate and a divalent cation. Li et al. have shown that during cell cycle TKTL1 is overexpressed in late G1 phase and S phases. This results in the formation of stable TKT/TKTL1-heterodimers which exhibit a different transketolase activity than TKT-homodimers. The formation of TKT/TKTL1 heterodimers shifts glucose metabolism towards R5P formation, resulting in an accumulation of R5P in the cell [Li et al. 2019; Coy JF 2017]. In addition, it has been shown in cancer cells that TKTL1 is involved in the regulation of cell division via a feed-forward loop mechanism, i.e., that the end product of the enzyme reaction further enhances the reaction [Sun W et al. 2010]. In terms of cell division, this binary procedure ("TKTL1 switch on/off"; TKTL1 on = shifts the glucose metabolism towards R5P formation) appears to be judicious: during the S phase, in which DNA duplication occurs in the cell, the DNA is unpackaged and accordingly extremely vulnerable to mutations. It is therefore essential to complete this phase as quickly as possible in order to minimize the risk of DNA damage. Thus, by means of a binary regulatory mechanism, the cell ensures that cell division - once it has begun - can proceed safely and completely and that sufficient building blocks (R5P) are available for DNA synthesis and DNA repair during this phase. These R5P building blocks are also required by RNA viruses for replication and form the basis for the replication of RNA viruses in the cell.

Since transketolases are essential for the formation of R5P and thus for the replication of SARS-CoV-2 in the host cell, they represent an attractive target for drug therapy: Substances that inhibit the activity of the transketolases prevent the accumulation of R5P, the essential building block for virus replication, in the cell. In this way, the virus can be deprived of its essential basis for its replication. Thus, this therapeutic approach, which fundamentally prevents viral replication - unlike a vaccine - is also independent of mutations of the virus.

Thiamine antagonists, such as oxythiamine (OT), are irreversible inhibitors of thiamine-dependent enzymes such as transketolases and efficiently inhibit sugar metabolism [Tylicki et al. 2018]. Since the TKT/TKTL1 heterodimer, which is responsible for enhanced R5P synthesis, is being dependent on thiamine, the use of OT or B-OT enables an inhibition of the TKT/TKTL1 heterodimer thereby reducing the R5P synthesis in a dose dependent manner. Thus, it can be assumed that ribose-5-phosphate can be reduced to a certain critical low level, where virus's replication is significantly slowed down or even no longer possible due to limitation of ribose-5-phosphate concentration in cells as a building block for virus replication.

Recent studies in SARS-CoV-2 infected Caco-2 cell lines [Bojkova D et al. 2020 and 2021] have shown that a) the pentose phosphate pathway was activated after SARS-CoV-2 infection and b) a dose-dependent inhibition of SARS-CoV-2 replication was detectable when non-toxic B-OT concentrations were applied.

6.2 Risk-Benefit and Ethical Assessment

B-OT represents a novel inhibitory thiamine (vitamin B1) analog and prodrug which is converted in the human body into oxythiamine. Oxythiamine is a thiamine antagonist and inhibits transketolase, the enzyme that controls the nonoxidative branch of the pentose phosphate pathway (PPP). Via the intermediate ribose-5-phosphate, the PPP supplies ribonucleotides for SARS CoV2 replication. Consequently, inhibition of transketolase by B-OT will reduce the capacity for viral replication. Subjects in this study should not expect to receive any benefit from the study intervention provided. If benfo-oxythiamine (B-OT) is shown to be safe and efficacious in future studies, there is potential benefit for future patients.

Based on the effects observed in the nonclinical studies, adverse effects can be detected in biochemistry and hematology parameters. Therefore, these parameters will be carefully checked in this study. Safety laboratory assessments, consisting of a complete blood count with differential (red and white blood cells), a comprehensive panel of coagulation (Fibrinogen, PT and APTT) and biochemistry (with a specific attention to liver function parameters) as well as a urinalysis, will be conducted prior to dosing (baseline), during treatment and at the end of treatment. Abnormal laboratory parameters will be followed-up until return to normal or baseline values.

Based on the safety results and PK results of single ascending dose part of this study as well as on the multiple dose non-clinical data, the overall risks for the subjects are considered to be small. The risks are further reduced by the chosen study design and risk mitigations.

For more information, please refer to the IB (Investigator's Brochure).

7 STUDY OBJECTIVES

Primary objective:

- To assess the safety and tolerability of single and multiple ascending doses of B-OT.

Secondary objectives:

- To evaluate the pharmacokinetics (PK) of benfo-oxythiamine (B-OT) and its active metabolite oxythiamine (OT) in plasma following single doses administered to healthy volunteers.
- To evaluate the pharmacokinetics (PK) of benfo-oxythiamine (B-OT) active metabolite oxythiamine (OT) in plasma following multiple doses administered to healthy volunteers.

Exploratory Objectives

- To evaluate the pharmacodynamics (PD) by measuring the transketolase activity
- To analyse PK parameters of other B-OT derivative metabolite(s), if any.

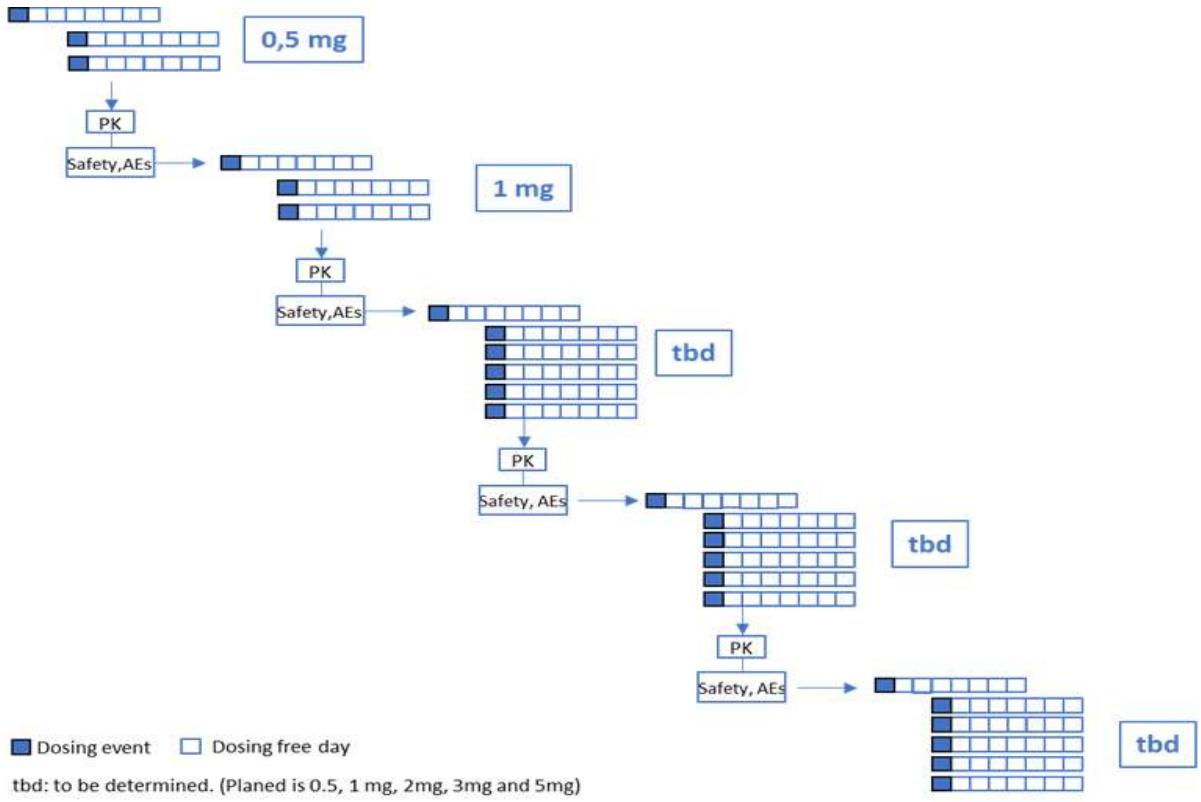
8 STUDY DESIGN

8.1 Overall Study Design and plan

This is a First-in-Human (FIH) single centre, open label study to assess safety, tolerability, pharmacokinetic and pharmacodynamic properties of benfo-oxythiamine (B-OT) after a single and multiple oral dose administration under fasting conditions in healthy male subjects.
This study has two parts: a single ascending dose (SAD) part and a multiple ascending dose (MAD) part.
The SAD part of the study will consist of an ambulatory screening visit, treatment period comprised of four nights (day -1 until day 4) in the clinical site where the study will be conducted during which safety parameters will be measured and numerous blood samples will be drawn for assessment of benfo-oxythiamine (B-OT) and oxythiamine PK and PD properties and a final ambulatory follow-up visit three days after subject's discharge (day 8). After the IMP administration during the treatment visit, safety, pharmacokinetic and pharmacodynamics data will be collected and reviewed 72 hours post-dose.

The following figure represents the study design of the SAD study:

Figure 1 SAD design



The filled box represents a dosing event, a white box a represents a dosing free day. Single dose treatment will be administered on Day 1.

The total duration of the SAD study for each subject will be up to 5 weeks divided as follows:

- Screening: Up to 28 days before the first day of the treatment visit;
- Treatment and study assessments: Admission in the clinical site on day -1 and discharge on day 4

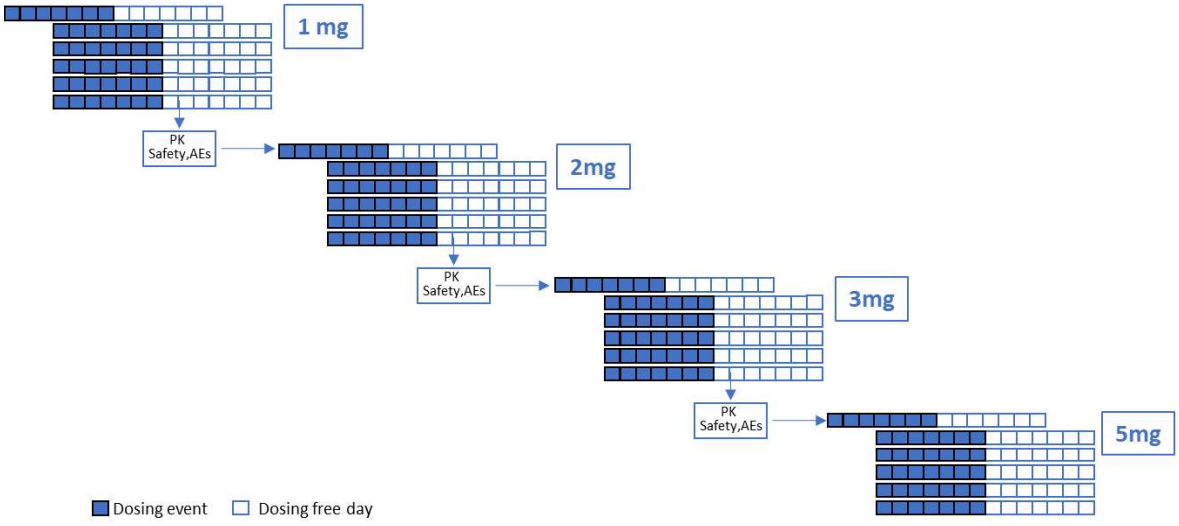
- Final follow-up visit (End of study (EOS)): three days after treatment visit completion on day 8.

The following description of activities during each study visit concerns each subject, regardless of whether it is the sentinel subject or not.
After confirmation of eligibility during the screening visit, each subject will undergo one treatment visit. The treatment visit will consist of five days (4 nights): on the first day subject(s) will come to the site for admission in the afternoon under the condition that a negative Sars-CoV2 PCR test has been confirmed after the performance of a test on the same day in the morning at the responsible laboratory location. On the second day (day 1) subject(s) will be administered the assigned IMP in the morning and the serial PK and PD sampling that has started pre-dose will continue post-dose until day 4 (72nd hour). On the fifth day (day 4) after the last PK sample (72 h post-dose) subject(s) will be discharged from the unit. During the treatment visit each subject will undergo not only serial PK and PD sampling but all assessments listed in the Schedule of Activities (Table 1).

A follow up visit will be held by each subject 3 days after the last day of the treatment visit during which they will complete their participation. All safety events that are reported as not resolved during the follow-up visit will be observed until resolution, but not longer than 30 days thereafter or after the end of the study (defined as last subject last visit).

In the MAD part of the study, B-OT will be administered orally (p.o.) once daily for 7 days (D1-D7), followed by 7 days without dosing. The first dose will be 1 mg, as the PK and safety data have shown a very low concentration of OT at 0.5 mg dose and no safety issues were observed at any dosage in the SAD part.
Each cohort will start with a sentinel subject (subject #1).
Subjects #2 - #6 of the same cohort will start treatment after a minimum of 72 hours if no adverse event is observed in the sentinel subject.

The following figure represents the study design of the MAD part:



The total duration of the MAD part of the study for each subject will be up to 6 weeks divided as follows:

- **Screening:** Up to 28 days before the first day of the treatment visit;

- confinement visit: Admission in the clinical site on Day -1, seven treatment days (Day1,2,3,4,5,6,7) and discharge on Day 8
- Ambulatory follow-up visit: four days after last treatment day on Day 11
- Final follow-up visit (End of study (EOS)): seven days after last treatment day visit on Day 14

After confirmation of eligibility during the screening visit, one treatment visit will follow. The confinement visit will consist of 9 days (8 nights): on the first day the subject will come to the site for the confinement period in the afternoon, under the condition that a negative Sars-CoV2 antigen test has been confirmed on the same day. Over the next seven days (Day 1 until Day 7) the subject will be administered the assigned IMP in the morning, and the serial PK and PD sampling that has started pre-dose will continue post-dose until Day 8. On Day 8, after the last PK sample, the subject will be discharged from the unit.

A follow-up visit will be held 4 days after the last day of treatment and a second follow-up during which the subject will complete their participation will be held seven days after the last day of the treatment visit. All safety events that are reported as not resolved during the follow-up visit will be observed until resolution, but not longer than 30 days thereafter.

A detailed description of all visits and procedures in the MAD part of the study is depicted in Table 2 Visit and Assessment Schedule of the MAD Part.

8.1.1 Screening (SAD and MAD)

During the screening visit, the study subjects will undergo a complete physical examination as well as ECG, vital signs (pulse rate, respiratory rate, blood pressure, body temperature), and relevant safety laboratory tests (hematology, biochemistry, urine analysis, viral screening) to assess and check if they meet the eligibility criteria as defined in sections 9.2 and 9.3. The screening is to take place within maximum 28 days and minimum 2 days prior to the first day (day -1) of the treatment visit.
All test results will have to be within acceptable (normal) ranges as set out in the CSP before a subject can be included in the study. Screening results that are outside the normal range will be assessed against the background of the subject's health status. The principal investigator or a delegated physician will then determine whether or not the respective abnormal test result is clinically significant and/or exclusionary before the subject can be enrolled in the study.

8.1.2 Treatment and observation period (SAD and MAD)

A total number of 24 subjects will be enrolled in the SAD part of the study in 5 cohorts:

Cohort 1 (0.5 mg B-OT): 3 subjects
Cohort 2 (1 mg B-OT): 3 subjects
Cohort 3 (2mg B-OT): 6 subjects
Cohort 4 (3 mg B-OT): 6 subjects
Cohort 5 (5 mg B-OT): 6 subjects

Each cohort will start with a sentinel subject (subject #1). Subject #2 - #3 or #2 - #6 of the same cohort will start treatment after a minimum of 48 hours if safety data indicate that dosing of subsequent subjects of this cohort is justified. Dose escalation to the next dose level will occur if three (first and second cohort) or six subjects (3 next cohorts) have completed the single dose treatment and if PK at Day 2 as well as safety data allow an escalation to the next dose level.

A total of 24 subjects will be enrolled in the MAD part of the study and assigned to multiple ascending dose groups in consecutive order:

Group 1 (1 mg B-OT): 6 subjects
Group 2 (2 mg B-OT): 6 subjects
Group 3 (3 mg B-OT): 6 subjects
Group 4 (5 mg B-OT): 6 subjects

Each cohort will start with a sentinel subject (subject #1). Subject #2 - #6 of the same cohort will start treatment after a minimum of 72 hours if safety data indicate that dosing of subsequent subjects of this cohort is justified. Dose escalation to the next dose level will occur if six subjects have completed the multiple dose treatment and if PK at Day 5 as well as safety data allow an escalation to the next dose level.

The health status of the trial subjects in the SAD and in the MAD part of the study will be assessed once again against the inclusion and exclusion criteria, as set by the study protocol, prior to study drug administration (Day -1). When considered suitable, the subjects will be assigned a unique study identifier and will be invited for treatment visit on day -1. Detailed description of all trial activities that need to be carried out are listed in the Visit and Assessment Schedule (Table 1 for the SAD part and Table 2 for the MAD part).

8.1.3 Follow-up (SAD and MAD)

Each subject in the SAD part of the study will complete the study with a final study visit which will take place on Day 8. This visit will include all procedures listed in the Visit and Assessment Schedule (Table 1).

Each subject in the MAD part of the study will visit the site for two follow-up visits – one on Day 11 and one end of study visit on Day 14. These visits will include all procedures listed in the Visit and Assessment Schedule (Table 2).

8.2 Justification for population and design

The study is based on established designs for FIH studies and will comprise of single ascending dose (SAD) and multiple ascending dose (MAD) cohorts in sequential order. It will be conducted in healthy volunteers to evaluate the safety, tolerability and PK, of oral benfo-oxythiamine (B-OT) administration.

The sentinel dosing maximizes the chance that, in the event of a significant unanticipated safety risk, dosing will be discontinued after exposure of a single healthy volunteers to a given dose level of benfo-oxythiamine (B-OT).

The sequential nature of the cohorts requires that the necessary data is reviewed by the SRC before the next cohort/sub-part/part can begin.

The study is to be conducted in healthy male subjects. 24 subjects represented in the SAD PK Analysis Set and another 24 subjects represented in the MAD PK Analysis Set will be required to complete the study. A healthy subject population with carefully considered inclusion/exclusion criteria will avoid the potential for interaction of benfo-oxythiamine (B-OT) with any underlying disease state or concomitant medication that might be necessary to administer to patients, while ensuring that subjects are fit and well enough for participation in the study.

The eligibility criteria are designed to select subjects for whom protocol treatment and procedures are considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject.

8.3 Proposed Safe Starting Dose

According to current CHMP recommendations in the "Guideline on Strategies to Identify and Mitigate Risks for First-in-Human and Early Clinical Trials with Investigational Medicinal Products" (EMEA/CHMP/SWP/28367/07 Rev. 1, July 2017), the first-in-human dose will be calculated considering results of the planned toxicology studies. No further considerations on the expected biological effective dose from completed pharmacology studies with B-OT were included, since efficacy studies have been primarily performed in rodents, which have been proven being less sensitive to B-OT compared to other species, including the dog. Therefore, considerations in the FIH dose are based on safety data only.

B-OT rapidly degraded in plasma, and high levels of the active metabolite oxythiamine were detected. The in vitro formation rate of the metabolite in rat and dog plasma was approximately two-fold higher than in human plasma. Plasma protein binding in human plasma is high (92.8% - 94.5%). B-OT is not well transported passively over Caco-2 cells or across the blood-brain barrier. The compound does not bind to red blood cells. B-OT as well as oxythiamine do not inhibit human CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 isoenzymes up to 200 µM (98 µg/mL B-OT or XX µg/mL OT). Two major metabolic pathways were found in in vitro metabolism studies with mouse, rat and human hepatocytes.

In both species tested (rat and dog), no plasma concentrations of B-OT could be measured in DRF studies at doses up to 1000 mg/kg in rats and 1 mg/kg in dogs, respectively. Therefore, in repeat dose toxicity studies only TK of the (main) metabolite oxythiamine was measured. In the repeat-dose study in rats the exposure (Cmax and AUC0-t) to oxythiamine was roughly dose-proportional. No major sex differences were observed nor accumulation in Cmax. AUC-values were similar or slightly under-proportional after repeated dosing. The toxicokinetic data from the repeat dose study in dogs showed a slightly under proportional increase of Cmax with increasing dose, while for AUC0-t linearly proportional increase was observed in males and apparently slightly over-proportional increase in females. No sex differences in exposure were observed. No difference in Cmax values were observed after multiple dosing, while for AUC no or a slight over-proportional increase was found (less than two folds accumulation after repeated doses).

The safety and tolerability of B-OT were assessed through a series of non-clinical toxicity studies, which included safety pharmacology investigations. The safety pharmacology investigations in rats (i.e., Irwin study) showed effects in female animals (1000 mg/kg/day) which were in a bad condition at the moment of the investigations. In the dog cardiovascular investigations, no effects on ECG by conventional ECG measurement. In a jacketed telemetry measurement, increases in heart rate were observed, at a supra-NOAEL dose level. In the same study no effect on respiratory functions were recorded.

Two-cycle (i.e., 1-week treatment followed by a 1-week treatment free period per cycle) repeat dose toxicity studies with B-OT were performed in rats and dogs. Female rats at the highest tested dose level (1000 mg/kg/day) were all prematurely euthanized on Day 7 of the study because of moribund condition. Other effects noted in the rats were lower body weight, transient hematological effects and some effects on cholesterol and triglyceride levels. In dogs one female animal was found dead on Day 5 in the second treatment cycle. Effects in surviving

animals were lower body weight and food consumption, which were partially reversed after a 1-week treatment-free period, as well as some transient changes in clinical pathology parameters. Furthermore, some non-adverse histopathological effects in thymus tissue were found. The No Observed Adverse Effect Level (NOAEL) for the rat study was set at 1000 mg/kg/day (males) and 300 mg/kg/day (females), and the NOAEL for the dog study was set at 0.15 mg/kg/day for both sexes.

Dosing is based on NOAEL x HED x 1/10 (No Observed Adverse Effect Level x Human Equivalent Dose [dog/human] x Safety Factor). As NOAEL was established in dog after a 7-day treatment and B-OT has already been used in compassionate use at a minimum daily dose of 3 mg for 7 days in 5 Covid-19 patients and 5 late-stage prostate cancer patients without any safety issue, only 3 subjects in the SAD part will receive the two first lowest doses.

In the SAD part, the first dose of B-OT will be 0.5 mg (0.0083 mg/kg body weight). After the first cohort, enrolment will be halted. If no safety issue is observed, the second dose level will be 1 mg (0.016 mg/kg body weight). The first and second dose level cohort will have 3 subjects. The human PK/PD model will be refined before enrolling to the next dose level may resume. As this is the first study testing B-OT in humans, study design adjustments may be made based on emerging data from each dose cohort based on review of preliminary safety, tolerability, and PK/PD results. If these data allow for, the dose escalation scheme of 2 mg, 3 mg and 5 mg will follow in 3 additional cohorts of 6 subjects.

Based on the SAD PK results, a starting dose of 1 mg in the MAD part provides a margin of exposure of 7.6 to steady-state exposure values observed at the NOAEL in dogs and is considered acceptable. If these data allow for, the dose escalation scheme of 2 mg, 3 mg and 5 mg will follow in 3 additional cohorts of 6 subjects.

For more information, please refer to the IB.

8.4 Dose Escalation Strategy

In general, a safety review committee (SRC) approval of commencement of a cohort is contingent on acceptable safety, tolerability, and PK results.

Each cohort will start with a sentinel subject (subject #1). The rest of the subjects of the same cohort will start treatment after a minimum of 48 hours in the SAD part or 72 hours in the MAD part if safety data indicate that dosing of subsequent subjects of this cohort is justified.

In the SAD part of the study, dose escalation to the next dose level will occur if three (first and second cohort) or six subjects (3 next cohorts) have completed the single dose treatment and if PK as well as safety data allow an escalation to the next dose level.

In the MAD part of the study, dose escalation to the next dose level will occur if six subjects (each of the 4 cohorts) have completed the multiple dose treatment and if PK as well as safety data allow an escalation to the next dose level.

The general dose increase is planned to follow a modified Fibonacci scheme. It may be adapted to the PK and safety profile analysis. However, a maximum multiplication factor of 3 will not be used.

Each cohort, after the first, may only commence after SRC review of results from the preceding cohort.

8.5 Stopping Criteria for Dose Escalation

Dose escalation will not continue to the next dose level if (a) maximal OT plasma levels are achieved, i.e. further increase is not justified from a scientific point of view, or if (b) toxicities are observed that do not justify continuation from an ethical point of view.

The study will be halted if one or more subjects experience a serious adverse event (SAE) that is considered as related to B-OT or 2 or more subjects in the same group experience severe AEs that are considered to be related to B-OT.

Dose-escalation will stop if clinically relevant signs or symptoms of similar nature occur in 2 or more subjects in a group that, in the opinion of the PI, warrant stopping of dose-escalation.

8.5.1 Stopping Rules for an Individual Subject, at any Time in the Study

Dosing for any individual subject will be stopped if the subject experiences a possible drug-related SAE or a possibly drug-related significant non-serious AE, which in the opinion of the SRC or investigator warrants discontinuation of the subject from the active protocol for his or her well-being.

8.5.2 Stopping Rules for a Whole Cohort, Dose Escalation, Progression to Next Study Part and Termination of Study

Stopping rules as detailed below are applicable for each of the following situation:

- Stopping dosing for a whole cohort.
    - When subjects in a cohort are dosed staggered.
- Stopping dose escalation.
- Stopping progression to the next part of the study.
- Final stop of study.

The study will be halted (i.e., no further dosing will occur) if one or more subjects experience an SAE that is considered to be related to B-OT or 2 or more subjects in the same group experience severe AEs that are considered to be related to B-OT. If, following an internal safety review, the Sponsor deems it appropriate to restart the study, this can be done following approval of a substantial protocol amendment.

Dose-escalation will stop if clinically relevant signs or symptoms of similar nature occur in 2 or more subjects in a group that, in the opinion of the PI, warrant stopping of dose-escalation.

The risk to all participating subjects will be evaluated thoroughly prior to a decision to terminate the study prematurely or continue dosing in agreement with the regulatory authorities.

The SRC will carefully review the totality of available data, taking into account moderate non-serious AEs at least possibly related to IMP administration in unblinded fashion, the number of subjects in which they occur, concurrency of more than one within the same subject and potential safety signals identified for other IMPs in the same class (mechanistic and/or chemical). Although changes outside normal ranges that apply for healthy volunteers are most relevant, changes from baseline measurements will also be considered observing any trends.

8.6 Safety Review

The SRC will consist of the following core members:

Principal investigator, sponsor safety representative, sponsor head of clinical department, sponsor PM.

The SRC may also request to have attendance of or off-line support and input from the following functions as required: CRO PM, sponsor preclinical department representative, CRO statistician and PK analyst.

Written statements and conclusions by the SRC will be in place before allowing study progression at the noted times as per CSP. This includes documentation of appropriate quality control checks on the data reviewed.

8.6.1 Data Reviewed by Safety Review Committee for Dose Escalation Decision

The SRC will decide on the next dose levels throughout the study, considering at each decision point the degree of uncertainty. The choice of the dose levels will take into consideration an estimate of exposure levels to be achieved and potential adverse effects. Emerging Cmax and AUC data will always be benchmarked to exposures expected at key human predicted doses (PAD/ATD). Late emerging safety issues that may have occurred after the time point for the dose escalation decision will always be considered.

In the SAD part of the study, at every SRC meeting, cumulative data for all cohorts will be reviewed. The following data will be reviewed for each individual cohort:

- Safety and tolerability data for 4 days for at least 3 healthy volunteers in the 2 first cohorts and 5 healthy volunteers in the next cohorts.
- PK data for 48 hours for at least 3 or 5 healthy volunteers in the first or next cohorts respectively.

Appropriate data collection up to 7 days in a given dosing cohort should be complete to proceed to the next dose cohort.

In the MAD part of the study, at every SRC meeting, cumulative data for all cohorts will be reviewed. The following data will be reviewed for each individual cohort:

- Safety and tolerability data for 8 days for at least 5 healthy volunteers in each cohort.
- PK data for 96 hours for at least 5 healthy volunteers in each cohort.

Appropriate data collection up to 14 days in a given dosing cohort should be complete to proceed to the next dose cohort.

8.6.2 Safety Review Committee Decision on Next Dose

The SRC will take the decision for the next dose level or whether to stop the study after reviewing all the pertinent safety and any other relevant data.
If consensus among the voting SRC members cannot be reached then the PI, who has the ultimate responsibility for the safety of the healthy volunteers, will make the final decision on the next dose level or whether to stop the study. In any event, dose escalation can only occur if agreed by the PI.
The decisions of the SRC on the next dose level will be documented and provided to all the appropriate parties involved with the study, including the Pharmacist to enable IMP preparation for the next scheduled dosing day.

- Where stopping criteria have not been met, the decision of the SRC may be to give the next higher dose according to the predefined dose increment, a smaller dose than previously given, a repeated dose or to stop dosing.
- A dose in which the safety stopping criteria have been met will not be repeated and further dose escalation **must not** occur.
  - In this case, the SRC will review the totality of data and restart of dosing is possible without a substantial amendment (e.g., in the case of a laboratory error) if SRC review concludes that the relevant stopping criterion was not fulfilled.
  - A lower dose level expected to be tolerable and not to meet the stopping criteria, would be acceptable in this case.
  - If the safety stopping criteria are met and the SRC decides that there are reasons that the dose level should be repeated or further dose escalation is warranted a summary of the data and justification (CSP amendment) will be submitted to the ESB, the Regulatory Authority and the IEC/IRB for their approval before further dosing.
- In some circumstances, consideration can be given to test fractionated or split doses.

8.6.3 Blinding at Safety Review Committee Meeting

Data will be reviewed unblinded.

8.6.4 Assessments Adaptation

Following review of data from a cohort of healthy volunteers, the timing of assessments and/or blood samples may be adjusted for subsequent cohorts.
Additional assessment or sampling times may be added if indicated by the data; however, the maximum blood volume taken from each healthy volunteer will not exceed 500 mL.

9 STUDY POPULATION

9.1 Subject population

Subjects will be recruited from the subject database of the investigational team in the Clinical site as well as through referrals.
Subjects who fail to meet the inclusion criteria or meet any exclusion criterion should not, under any circumstances, be included into the study. There can be no exceptions to this rule. If subjects fail out of the screen window but did not fail on any inclusion or exclusion criteria, they can be rescreened. One repeat assessment may be requested per the discretion of investigator at the screening visit and on admission to the Clinical site for the treatment visit.

9.2 Inclusion criteria

The following criteria must be met by all subjects considered for study participation:

1. Signed and dated informed consent form
2. Subjects capable to understand the purposes and risks of the study, and who are willing and able to participate in the study
3. Male volunteers who are willing to comply with contraception requirements
4. Aged 18-60 years, inclusive
5. Healthy participants, as determined by screening assessments and Principal Investigator's judgment. Healthy status is defined by the absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECG, hematology, blood chemistry, serology and urinalysis
6. Body Mass Index (BMI) of 18-30 kg/m^2 inclusive
7. Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame:
    - Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
    - Use a male condom plus partner use of a contraceptive method with a failure rate of < 1% per year when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.

Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

9.3 Exclusion Criteria

Subjects will be excluded if they meet any of the following criteria:

1. Subjects who have a clinically relevant history as determined by the Investigator, or presence of respiratory, gastrointestinal, renal, hepatic, hematological, lymphatic, neurological, cardiovascular, psychiatric, musculoskeletal, genitourinary, immunological, dermatological, endocrine, connective tissue diseases or disorders.
2. Fridericia's correction factor for QT (QTcF) > 450 ms or history of QT interval prolongation.
3. Have acute gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, heartburn) at the time of screening or admission.
4. Has any abnormal laboratory value of Grade 2 or higher, which is considered as clinically significant by the PI (or designee).

5. Has values of ≥ 10 % above the upper limit of normal for the following laboratory analytes:
    - alanine aminotransferase (ALT/SGPT), alkaline phosphatase (serum), aspartate aminotransferase (AST/SGOT), at Screening and Day-1
    - creatinine and urea concentration in blood at Screening and Day-1
6. Have a clinically relevant surgical history, as determined by the Investigator.
7. Have a history of relevant drug hypersensitivity.
8. Have a history of alcoholism or drug abuse.
9. Have a significant infection or known inflammatory process at screening or admission.
10. Have used any prescription or non-prescription medicines within 2 weeks of admission, unless in the investigator's opinion it will not affect the determination of safety or other study assessments.
11. Have received any investigational drug within 30 days prior to screening.
12. Have used tobacco or nicotine products within 3 months of screening
13. Has a positive alcohol or drug screen at Screening or the Day 1 visit
14. Have donated or received any blood or blood products within the 3 months prior to screening.
15. Cannot communicate reliably with the investigator.
16. Are unlikely to co-operate with the requirements of the study.
17. Have been vaccinated with a Covid-19 vaccine within 2 weeks prior to screening and/or during participation in the clinical trial

Abnormal vital signs after 5 minutes supine rest, are defined below. Abnormal values may be repeated once at the discretion of the investigator:

- Systolic BP < 90 mmHg or > 140 mmHg
- Diastolic BP < 50 mmHg or > 90 mmHg
- Pulse < 45 or > 85 bpm
- Body temperature of > 37.7°C (on admission day)

Clinically important abnormalities in rhythm, conduction or morphology of the resting ECG and any clinically important abnormalities in the 12-lead ECG are defined below:

- Prolonged QTcF > 450 ms.
- Family history of long QT syndrome.
- PR (PQ) interval shortening < 120 ms (PR > 110 ms but < 120 ms is acceptable if there is no evidence of ventricular pre-excitation).
- PR (PQ) interval prolongation (> 240 ms) intermittent second (Wenckebach block while asleep is not exclusive) or third-degree AV block, or AV dissociation.
- Persistent or intermittent complete BBB, IBBB, or IVCD with QRS > 110 ms. Volunteers with QRS > 110 ms but < 115 ms are acceptable if there is no evidence of eg, ventricular hypertrophy or pre-excitation.

9.4 Prior and concomitant medication

Prior Medication: All prior medications (prescription and over-the-counter [OTC]) taken within 90 days of study screening will be recorded. Prescription or non-prescription drugs, including vitamins, herbal and dietary supplements should not be taken within 14 days (or 5 half-lives, whichever is longer) prior to the first dose of Benfo-oxythiamine (B-OT), unless in the opinion of the Investigator and Sponsor the medication will not interfere with the study procedures or would compromise subject safety. Prescription or non-prescription drugs,

including vitamins, herbal and dietary supplements taken during the 14 days before the first dose of benfo-oxythiamine (B-OT), and the reason for taking them, will be noted in the subject's CRF.

Inclusion of subjects who have taken prior medication will be reviewed on a case-by-case basis in relation to the safety aspects and objectives of this study.

Concomitant Medication: Prescription or non-prescription drugs, including vitamins, herbal and dietary supplements should not be taken throughout the duration of the study, with the exception of paracetamol (which may be taken as an analgesic to a maximum of 2 g in 24 h (500 mg 4 times a day) and ibuprofen (which may be taken as an analgesic to a maximum of 1.2 g in 24 h (400 mg 3 times a day).

Medication, which is considered necessary for the subject's safety and well-being, may be given at the discretion of the investigator.

When any medication is required, it should be prescribed by the investigator. Following consultation with benfovir AG, the investigator should determine whether or not the subject should continue in the study. Administration of concomitant medications that may influence the measurement of the PK and PD endpoints may be documented as a protocol deviation after consultation of the investigator with benfovir AG.

If intake of ANY prior or concomitant medication is necessary during the study, the daily dosage, duration and reasons for administration will be recorded on the subject's CRF.

9.5 Dietary and other restrictions prior and during the study

To avoid possible negative effects on the measurement of plasma drug concentrations and/or on subject's safety, several restrictions are to be adopted as outlined in the eligibility criteria and in the following points.

1. Subjects should abstain from alcohol for 72 hours prior to check-in at the Clinical site and before their final Follow-up Visit. Subjects should consume no more than 2 units of alcohol per day during the ambulatory days of participation in the study.
2. The consumption of xanthine-containing products including coffee, tea, cola, energy drinks or chocolates is not allowed 24 hours prior to check-in at the Clinical site until discharge.
3. For consistency, all subjects will receive a light meal immediately prior to commencing a 10 hour fast before dosing. During the fast, water will be freely available, except during the period 1 hours before dosing to 1 hours after dosing. During this 2-hour period only the 240 mL of water required for dosing will be available. Decaffeinated tea and coffee are allowed from 4 h post-dose. The subjects will be served standardized meals throughout the confinement in the site as follows:
    - Subjects will fast overnight for at least 10 hours before dosing.
    - Lunch will be served: approximately 4 h post-dose.
    - Snack will be served: approximately 8 h post-dose.
    - Dinner will be served: approximately 12 h post-dose.
4. Poppy seed or foods containing poppy seeds are not permitted from 3 days before drug of abuse test, as consumption can lead to a positive opiate result in the drugs of abuse test.

5. No food or drink containing grapefruit, cranberry, or Seville oranges (including marmalade and fruit juices), and/or food or drink, sweets, candies or other confectionary containing liquorice will be allowed from 7 days prior to check-in at the Clinical site until the final study visit.
6. Smoking is not allowed during the participation in the clinical study.
7. Strenuous physical activity (e.g., heavy lifting, weight or fitness training) is not allowed within 72 hours prior to check-in day and until the final follow up visit.
8. Subjects will remain seated or ambulatory for the first 4 hours following study treatment administration. However, should adverse events occur at any time, subjects may be placed in an appropriate position.
9. Subjects will be advised that they should not donate blood for at least 3 months after the final study visit.
10. Subjects must not donate sperm for at least 3 months after dosing with benfo-oxythiamine (B-OT).
11. During subjects' ambulatory periods, they should abstain from consuming high energy drinks (e.g., Red Bull®), and food containing poppy seeds and any OTC medication or herbal preparations until after their final Follow--up Visit has been completed. Subjects should also limit their caffeine intake to equivalent of 3 servings of coffee per day (in the US: 1 serving = 12 oz soda, 6 oz coffee, or 8 oz tea; in the UK: 1 serving = 330 mL cola, 180 mL coffee, or 240 mL tea).

During each period of the study, bathrooms will be locked for at least 4 hours after each drug administration. If necessary, subjects will be permitted to use the washroom facilities under supervision only during this interval. Each subject will be questioned on the specific points at admission to the clinical site. If a subject admits a non-compliance with these restrictions, the Principal Investigator will decide whether or not the subject will be permitted to remain in the study. Non-compliance with these restrictions will be noted in the subject's source documents.

9.5.1 Contraception requirements

There is no information about effects that B-OT could have on the development of the foetus in humans. Therefore, it is important that WOCBP, who are the partners of male subjects, do not become pregnant during the study and for a total period of 3 months after the male subject has attended the study Follow-up Visit.

Male subjects who have been sterilised are required to use one barrier method of contraception (condom) from the time of IMP administration until after the follow-up visit. The subject must have received medical assessment of the surgical success.

As a precaution, all non-sterilised male subjects should avoid fathering a child by either true abstinence* or use a condom and their female partner/spouse has to be either of non-childbearing potential or has to use a highly effective contraception form of birth control, starting from the time of IMP administration until 3 months after the study Follow-up Visit. The female partner/spouse should be stable on their chosen method of birth control for at least 3 months prior to first dosing with the IMP.

* Sexual abstinence is defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. It is only acceptable if preferred and usual lifestyle of the subject.

Highly effective contraception, i.e., a form of birth control with a failure rate of less than 1% per year when used consistently and correctly, which are allowed in this clinical study, are:

- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:

- Oral
- Intravaginal
- Transdermal

- Progestogen-only hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Injectable
    - Implantable
    - Intrauterine device
    - Intrauterine hormone-releasing system
    - Bilateral tubal occlusion

In addition, a barrier method must also be used i.e., condom (without spermicidal foam/gel/film/cream/suppository or fat- or oil-containing lubricants) or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.

Male subjects will be instructed that if their partner becomes pregnant during the study this should be reported to the investigator. The investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study. In the event that a subject's partner is subsequently found to be pregnant after the subject is included in the study, then consent will be sought from the partner and if granted any pregnancy will be followed and the status of mother and/or child will be reported to the sponsor after delivery.

A pregnancy notification form and follow-up will be completed.

9.6 Study drug discontinuation and subject withdrawal

9.6.1 Study drug interruption or discontinuation

Before IPM is administered, changes in the subject health status including laboratory results if applicable, since the previous visit has to be checked. The investigator must temporally interrupt or permanently discontinue study participation if continued study participation is believed to be contrary to the best interests of the subject. The interruption or premature discontinuation of study participation might be triggered by:

- Lost to follow-up.
- Any SAE that is considered related to study intervention by the investigator.
- Any other AE that, in the opinion of the investigator or the sponsor, warrants discontinuation of further dosing of study intervention.
- Pregnancy.
- Diagnostic or therapeutic procedure
- Clinically significant abnormal assessment (e.g., ECG, vital signs or laboratory abnormalities)
- Administrative reasons in particular withdrawal of the subject's consent and/or severe non-compliance to the CSP.

If the study is permanently discontinued, the subject will remain in the study to be evaluated for assessments at the follow-up visit, including follow-up of any AEs, unless consent is

withdrawn from further study participation, the subject is lost to follow-up, or the subject is enrolled in another clinical study.

The reason for interruption or premature discontinuation of study participation must be documented in the source documentation and on the Case Report Form and reported to the monitor.

9.6.2 Subject withdrawal

Subjects have the right to withdraw from the study at any time for any reason. Should a subject decide to withdraw from the study, all efforts should be made to complete and report the observations, particularly the follow-up examinations, as thoroughly as possible.

The clinical investigator may withdraw the subject from the study for one of the following reasons:

- Positive answer to the re-assessment of any exclusion criteria, during the admission at the treatment visit or in a subsequent occasion;
- Non-adherence to the protocol requirements;
- Adverse events, symptoms or signs of possible toxicity;
- Any disease requiring medication;
- Any other condition that, according to the judgment of the investigator, is important for maintaining the health of the subjects;
- Vomit after administration and/or within 2 hours after study product administration;
- Diarrhoea within the dosing interval (2 hours prior, during dosing, 2 hours post dosing).

A subject who considers withdrawing from the study must be informed by the investigator about modified follow-up options (e.g., telephone contact, a contact with a relative or treating physician, or information from medical records).

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

If a subject withdraws from the study, it should be confirmed if he/she still agrees for existing samples to be used in line with the original consent. If he/she requests withdrawal of consent for use of samples, destruction of any samples taken and not tested should be carried out in line with what was stated in the ICF and local regulation. The investigator must document the decision on use of existing samples in the Clinical site study records and inform the Sponsor.

The details and reasons of the withdrawal from the study will be registered in the source documentation and on the Case Report Form and reported to the monitor.

A subject will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visit and is unable to be contacted by the Clinical site. The following actions must be taken if a subject fails to return to the Clinical site for a required study visit:

- The Clinical Unit site must attempt to contact the subject and reschedule the missed visit as soon as possible (if applicable) and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local

equivalent methods). These contact attempts should be documented in the subject's medical record.

9.6.3 Replacement policy

Subjects who have given informed consent may be replaced only prior to the beginning of the first drug administration. No replacement will be allowed thereafter.

10 INVESTIGATIONAL PRODUCT

10.1 Investigational drug and comparative drug

10.1.1 Investigational test drug

The investigational test product in this study is called benfo-oxythiamine (B-OT).

The active substance is benfo-oxythiamine monosodium and is formulated as powder-in-capsule.

For the present first-in-human clinical trial of B-OT, a hard gelatine capsule was selected as dosage form. Due to the low strength of the IMP of 0.1 mg, 0.5 mg, 3 mg, and 10 mg, excipients were added to facilitate filling of the capsule with equal amounts of active substance. The standard compendial excipient microcrystalline cellulose was added to the formulation as filler as well as some colloidal anhydrous silica as glidant and magnesium stearate as lubricant

B-OT is hygroscopic and capsule and container closure system have been selected to provide protection from humidity. B-OT capsules should be stored at room temperature inside the original primary packaging.

The manufacturer responsible for production, the primary and secondary packaging, batch control and release of test investigational medicinal product and for labelling of the IMP is benfovir AG, Germany.

10.1.2 Investigational comparative drug

Not applicable.

10.2 Study drug up- and down-titration

Please refer to section 6.2.8 Dose Escalation Strategy.

10.3 Packaging and labelling

Test investigational medicinal products packed for each subject will be prepared, labelled and supplied by the Sponsor. The Test IMPs will be packed and labelled by responsible persons delegated by the Sponsor according to Annex 13 of GMP.

The release of the IMP after packaging and labelling will take place in benfovir AG. The IMP can be released by the Sponsor once scanned copies of both an Ethics Committee (EC) and the Competent Authority (CA) approvals are received by the Sponsor.

The Test product will be provided as one bottle for each subject labelled with details of the product.

The containers will be labelled in accordance to the applicable regulatory requirements.

The Sponsor will provide sufficient quantity of the IMPs for administration and reserve purposes (to be used i.e. in case of damage or fall of tablets).

Labelling of these reserved products will be identical to the original one except for Subject Number and Period Number which is not written and should be completed by Investigator in case these reserved products will be used. The Certificates of Analysis for the Test products will accompany the IMPs and will be presented in the final study report.

Investigational Medicinal Products will be sent to clinical site under temperature monitoring conditions. Temperature monitoring data recorded during IMPs shipment will be evaluated by Sponsor. IMPs will be used in the study only after written Sponsor approval.

Upon arrival at the clinic, the investigational products should be checked for damage and proper identity, quantity, integrity of seals and temperature conditions should be verified, any deviations or product complaints upon discovery should be reported. All study drugs will be dispensed by the investigator or a person under his supervision. The subjects will not be given study medication to take home with them. Study drug packaging will be overseen by the Investigator or Investigator's designee and bearing a label with the identification according to the requirements of local law and legislation. All drug supplies must be stored in a secure, temperature-controlled area with limited access. For batch-specific storage instructions, see the packaging.

The test IMPs will be stored and administered by a medical professional, who is a member of the investigational team and in accordance with section 10.6.

10.4 Drug accountability

The study drug provided for this study will be used only as directed in the study protocol.

The study personnel will account for all study drugs dispensed to and returned from the healthy volunteer.

At the end of the study, study site personnel will account for all study drugs received at the site, unused study drugs and for appropriate destruction. Destruction must not take place unless the Sponsor has approved it. Certificates of delivery and destruction must be signed. The investigator is responsible for ensuring that dosing is administered in compliance with the protocol. Delegation of this task must be clearly documented and approved by the investigator. All study drug administration will occur under medical supervision.

10.5 Treatment assignment and blinding

10.5.1 Randomization and treatment assignment

This is a non-randomized study.

If a subject, who does not meet the selection criteria, has been dosed before the error is identified, the subject should be advised to continue safety assessments to ensure their safety. The investigator will inform the Sponsor of the error and a joint decision made as to whether the subject should be replaced.

10.5.2 Blinding

This is an open label study

10.6 Dosing Procedure

After an overnight fasting of at least 10 hours, the IMP will be administered to the subjects according to the dosing schedule for the cohort they are part of.

Dosing of the subjects will be conducted in the following manner: subjects will be dosed one by one in a designated room. Prior to each subject being invited to the dosing room, the PI and a designated study nurse will check the prepared IMP in accordance with the relevant dosing scheme. Upon subject's entry in the dosing room, the subject's number will be crosschecked with the IMP that is prepared and with the dosing scheme again. Upon confirmation by the PI and the study nurse that the IMP is the right one and can be administered, the PI will perform the administration according to the available instructions. The Investigator will also perform a visual IMP identity and intactness check of the IMP prior to the administration. In case of an incorrect identity or damaged tablet, the IMP will be replaced with a correct identity or undamaged tablet. IMP will be administered orally to each subject in an upright position. IMP

administration of the tablet will be done by a dispensation spoon followed by intake of 240 mL water as described in sections 10.6.1 – 10.6.3.

After the IMP was administered the PI will check the subject's oral cavity by looking under the tongue, under the lips, in the corners of the mouth and between gums and cheeks, using a tongue depressor or a spatula. In case of the Test IMP the PI will confirm that the subject had swallowed the administered dose.

Drug accountability and dispensing records will be maintained at all times. Each activity including date and time of each dose will be documented at the time it is performed in the source documentation. Copy of labels will be stuck in the source documentation at the time of dosing. All empty IMP containers/packaging will be stored in a secure area.

For each subject, all scheduled post dose activities and assessments will be performed relative to the time of study treatment administration.

For Test Product only the taste assessment will be done by the trained study personnel after dosing in each period and will be recorded in the source data form.

10.6.1 Benfo-oxythiamine (B-OT)

- Depending on the assigned dose, one or more than one tablet of the test product (benfo-oxythiamine (B-OT)) will be administered with 240 mL of water.

The tablet(s) must be swallowed whole. The tablet(s) must not be sucked, chewed or broken.

No water will be allowed one hour before and one hour after administration, except 240 mL of water administered together with benfo-oxythiamine (B-OT) administration.

10.7 Treatment Compliance

Administration of investigational products will only be performed by authorized clinical research staff and will be followed by a hand and oral cavity check in order to confirm the swallowing of the product and fluid intake, if applicable.

Upon dispensing medication, the clinical research staff will record the related information on the applicable source documents, to allow investigational product accountability and evaluation of subject compliance.

11 STUDY ENDPOINTS

11.1 Primary endpoints

The outcome measures for the safety endpoints of the study will be safety laboratory assessments, physical examination (PE), electrocardiograms (ECGs), vital signs, adverse events (AEs) and serious adverse events (SAEs).

The safety variables will mainly be addressed by the adverse events (AEs) data as determined by clinical or biochemical/ hematologic investigations.

The safety variables will be tabulated for all study participants. AE data will be processed in the statistical analysis after coding according to MedDRA dictionary. AEs will be presented in summary tables. These tables will show the number of subjects per group presenting an AE and the incidence of its occurrence. AEs will be grouped by system organ class and stratified by severity and by relation to study treatment.

Adverse events which will be summarized and will be categorized in subsets of all treatment-emergent AEs, and of all treatment-related AEs.

Clinical laboratory and vital signs will be summarized by change from baseline.

11.2 Secondary endpoints

In the SAD part of the study, the following PK endpoints will be derived from plasma benfo-oxythiamine (B-OT) and oxythiamine (OT) concentration versus time data following administration according to the dosing schedule: Cmax, tmax, t1/2, CL/F, λz, Vz/F, AUC0-inf and AUC last as appropriate.

In the MAD part of the study the following PK endpoints will be derived from plasma oxythiamine (OT) concentration versus time data following administration according to the dosing schedule on Day 1: AUC_{τ}, C_{max}, t_{max}, on Day 7: AUC_{τ}, $C_{max,ss}$, $t_{max,ss}$, Swing, PTF%, CL_{ss}/F, V_z/F, C_{trough}, C_{av}, C_{min}, $t_{1/2}$, AUC_{inf} and AUC_{last} as appropriate and Day7/Day1: $Ra(AUC_{\tau})$, $Ra(C_{max})$.

11.3 Exploratory endpoints

- To evaluate the pharmacodynamics (PD) by measuring the transketolase activity.
- To analyse PK parameters of other B-OT derivative metabolite(s), if any.

12 STUDY ASSESSMENTS (SAD AND MAD)

12.1 Visit Procedures (SAD)

The procedures per visit to be performed in the SAD part of the study are listed below.

12.1.1 Procedures during the Screening Visit (Day -28 to -2)

- Subjects will come to the Clinical site for an ambulatory screening visit
- Subjects will be fully informed verbally and in writing about the study procedures and their responsibilities/rights while participating in the study
- If the subject agrees to participate in the study, he or she will sign a written informed consent
- Subjects will provide nasopharyngeal and oropharyngeal swab which will be tested for the presence of SARS-COV-2 (COVID – 19) infection with a rapid antigen test
- Subjects will be screened for inclusion and exclusion criteria
- The Investigator will record any prior and concomitant medical conditions/medications
- Subjects will provide a detailed medical history and demographic data
- Subjects will undergo a complete physical examination including documenting weight, height, BMI
- Subjects will have their vital signs (heart rate, blood pressure, respiratory rate, body temperature) recorded
- Subjects will undergo a breath alcohol test
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, serology, urinalysis); the urine sample will be used also for drug screen test and cotinine test performed via dip stick
- Subjects will undergo a 12-lead ECG
- The Investigator will record any AEs that have occurred during visit with the subject
- Eligible subjects will be invited for a treatment visit

Subjects who meet the inclusion and have no exclusion criteria will be scheduled to attend the treatment visit (Day -1 to Day 4).

The subjects will arrive at the study site in the morning of Day -1 for the conduct of the treatment visit, provided that they have had a negative Sars-CoV2 tests result from the morning of the same day or the evening of the previous one. In case this time frame cannot be respected, the time of testing will be decided on a case-by-case basis by the Sponsor, respecting the official validity period of the test which is 72 h.

12.1.2 Procedures on Day -1

- Subjects will provide nasopharyngeal and oropharyngeal swab which will be tested via PCR for the presence of SARS-COV-2 (COVID – 19) infection in the morning by visiting the laboratory point of testing indicated by the Investigator during the screening visit
- Subjects will arrive at the unit in the afternoon after a confirmed COVID-19 negative result is available from the laboratory
- Subjects´ eligibility will be re-checked and medica history re-reviewed

- Subjects will undergo a complete physical examination including documenting weight
- The Investigator will review any AEs that have occurred since the previous visit with the subject
- Subjects will undergo a breath alcohol test
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, urinalysis); the urine sample will be used also for drug screen test and cotinine test performed via dip stick
- Subjects will have their vital signs measured
- The Investigator will record any changes in concomitant medications or illnesses with the subject since the last study visit
- Subjects will be admitted to the stationary unit of the site
- Subjects will receive unified balanced evening meal

12.1.3 Procedures on Day 1

- Subjects will be fasted from midnight (at least 10 hours fasting prior to dosing)
- Subjects will have their vital signs recorded (pre-dose and approximately 2, 4, 8, 12 hours after dosing)
- Subjects will undergo limited physical examination to target any change from the previous status or any body system deemed pertinent to that subject by the PI (or designee) 4 hours post dose
- Subjects will undergo a 12-lead ECG pre-dose
- Subjects will receive a single dose of the assigned IMP Benfo-oxythiamine (B-OT) between a time interval of two hours between 07:00 am and 09:00 am.
- Catheter for blood sampling will be inserted and serial blood samples will be collected for PK and PD analysis according to the sample schedule in section 12.4.1 Blood samples collection
- AEs that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.1.4 Procedures on Day 2

- Subjects will be fasted from midnight (at least 10 hours fasting prior to safety laboratory samples collection)
- Subjects will have their vital signs recorded
- Subjects will undergo limited physical examination to target any change from the previous status or any body system deemed pertinent to that subject by the PI (or designee)
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, urinalysis);
- Subjects will undergo a 12-lead ECG

- Blood samples will be collected for PK and PD analysis according to the sample schedule in section 12.4.1 Blood samples collection
- AEs that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.1.5 Procedures on Day 3

- Subjects will be fasted from midnight (at least 10 hours fasting prior to safety laboratory samples collection)
- Subjects will have their vital signs recorded
- Subjects will provide urine samples for laboratory urine analysis
- Blood samples will be collected for PK and PD analysis according to the sample schedule in section 12.4.1 Blood samples collection
- AEs that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.1.6 Procedures on Day 4

- Subjects will be fasted from midnight (at least 10 hours fasting prior to safety laboratory samples collection)
- Subjects will provide nasopharyngeal and oropharyngeal swab which will be tested via PCR for the presence of SARS-COV-2 (COVID – 19) infection
- Subjects will have their vital signs recorded
- Subjects will undergo limited physical examination to target any change from the previous status or any body system deemed pertinent to that subject by the PI (or designee)
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, urinalysis)
- Blood samples will be collected for PK and PD analysis according to the sample schedule in section 12.4.1 Blood samples collection
- AEs that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will be discharged from the Clinical site as soon as all scheduled procedures have been completed

12.1.7 Procedures on Follow-up visit (Day 8)

- Subjects will come to the Clinical site for an ambulatory End of study visit
- Subjects will undergo a complete physical examination including documenting weight
- Subjects will provide nasopharyngeal and oropharyngeal swab which will be tested for the presence of SARS-COV-2 (COVID – 19) infection with a rapid antigen test

- Subjects will have their vital signs (heart rate, blood pressure, respiratory rate, body temperature) recorded
- Subjects will undergo a 12-lead ECG
- Subjects will undergo a breath alcohol test
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, urinalysis); the urine sample will be used also for drug screen test and cotinine test performed via dip stick
- Blood samples will be collected for PK and PD analysis according to the sample schedule in section 12.4.1 Blood samples collection
- AEs that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will complete the study and their participation will be ended

See Table 1 for the time points of all the assessments planned in the study.

12.2 Visit Procedures (MAD)

The procedures per visit to be performed in the MAD part of the study are listed below.

12.2.1 Procedures during the Screening Visit (Day -28 to -2)

- Subjects will come to the Clinical site for an ambulatory screening visit
- Subjects will be fully informed verbally and in writing about the study procedures and their responsibilities/rights while participating in the study
- If the subjects agree to participate in the study, they will sign a written informed consent
- Subjects will provide nasopharyngeal (through the nose) and/or oropharyngeal (through the mouth) swab which will be tested for the presence of SARS-COV-2 infection with a rapid antigen test
- Subjects will be screened for inclusion and exclusion criteria
- The Investigator will record any prior and concomitant medical conditions/medications
- Subjects will provide a detailed medical history and demographic data
- Subjects will undergo a complete physical examination including documenting weight, height, body mass index (BMI)
- Subjects will have their vital signs (heart rate, blood pressure, respiratory rate, body temperature) recorded
- Subjects will undergo a breath alcohol test
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, serology, urinalysis); the urine sample will be used also for drug screen test and cotinine test performed via dip stick
- Adverse events (AEs) that have occurred during the visit will be recorded
- Subjects will undergo a 12-lead ECG
- Subjects will be invited for a treatment visit

If Subjects fulfill the inclusion criteria and do not meet any exclusion criteria, Subjects will be scheduled to attend the treatment visit (Day -1 to Day 8).

Subjects will arrive at the study site in the afternoon of Day -1 for the conduct of the treatment visit

12.2.2 Procedures on Day -1

- Subjects will provide nasopharyngeal and/or oropharyngeal swab which will be tested for the presence of SARS-COV-2 infection with a rapid antigen test
- Subjects will arrive at the unit in the afternoon
- Subjects´ eligibility will be re-checked and medica history re-reviewed
- Subjects will undergo a complete physical examination including documenting weight
- The Investigator will review any adverse events (AEs) that have occurred since the previous visit with the subjects
- Subjects will undergo a breath alcohol test
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, coagulation, urinalysis); the urine sample will be used also for drug screen test and cotinine test performed via dip stick
- Subjects will have their vital signs measured
- The Investigator will record any changes in concomitant medications or illnesses with the subjects since the last study visit
- Subjects will be admitted to the stationary unit of the site
- Subjects will receive unified balanced evening meal

12.2.3 Procedures on Day 1

- Subjects will be fasted from midnight (at least 10 hours fasting prior to dosing)
- Subjects will have their vital signs recorded (pre-dose)
- Subjects will undergo limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee) 4 hours post dose
- Subjects will undergo a 12-lead ECG pre-dose
- Subjects will receive a single dose of the assigned IMP (benfo-oxythiamine (B-OT) within a time interval of two hours between 07:00 am and 09:00 am.
- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) and pharmacodynamic (PD) analysis according to the sample schedule in section 12.4.1 Blood samples collection.
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.2.4 Procedures on Day 2

- Subjects will be fasted from midnight (at least 10 hours fasting prior to dosing)
- Subjects will have their vital signs recorded (pre-dose and approximately 4 hours after dosing)

- Subjects will undergo a limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee) 4 hours post dose
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, coagulation, urinalysis)
- Subjects will undergo a 12-lead ECG pre-dose
- Subjects will receive a single dose of the assigned IMP (Benfo-Oxythiamine (B-OT) within a time interval of two hours between 07:00 am and 09:00 am.
- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) and pharmacodynamic (PD) analysis according to the sample schedule in section 12.4.1 Blood samples collection.
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.2.5 Procedures on Day 3

- Subjects will be fasted from midnight (at least 10 hours fasting prior to dosing)
- Subjects will have their vital signs recorded (pre-dose)
- Subjects will undergo a limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee) 4 hours post dose
- Subjects will receive a single dose of the assigned IMP (Benfo-Oxythiamine (B-OT) within a time interval of two hours between 07:00 am and 09:00 am.
- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) analysis according to the sample schedule in section 12.4.1 Blood samples collection
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.2.6 Procedures on Day 4

- Subjects will be fasted from midnight (at least 10 hours fasting prior to dosing)
- Subjects will have their vital signs recorded (pre-dose)
- Subjects will undergo a limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee) 4 hours post dose
- Subjects body weight will be measured
- Subjects will undergo a 12-lead ECG pre-dose
- Subjects will receive a single dose of the assigned IMP (Benfo-Oxythiamine (B-OT) within a time interval of two hours between 07:00 am and 09:00 am.

- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) and pharmacodynamic (PD) analysis according to the sample schedule in section 12.4.1 Blood samples collection
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.2.7 Procedures on Day 5

- Subjects will be fasted from midnight (at least 10 hours fasting prior to dosing)
- Subjects will have their vital signs recorded (pre-dose and approximately 4 hours after dosing)
- Subjects will undergo a limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee) 4 hours post dose
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, coagulation, urinalysis)
- Subjects will receive a single dose of the assigned IMP (Benfo-Oxythiamine (B-OT) within a time interval of two hours between 07:00 am and 09:00 am.
- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) and pharmacodynamic (PD) analysis according to the sample schedule in section 12.4.1 Blood samples collection
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.2.8 Procedures on Day 6

- Subjects will be fasted from midnight (at least 10 hours fasting prior to dosing)
- Subjects will have their vital signs recorded (pre-dose)
- Subjects will undergo a limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee) 4 hours post dose
- Subjects will receive a single dose of the assigned IMP (Benfo-Oxythiamine (B-OT) within a time interval of two hours between 07:00 am and 09:00 am.
- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) analysis according to the sample schedule in section 12.4.1 Blood samples collection
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.2.9 Procedures on Day 7

- Subjects will be fasted from midnight (at least 10 hours fasting prior to dosing)
- Subjects will have their vital signs recorded (pre-dose and approximately 4 hours after dosing)
- Subjects will undergo limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee) 4 hours post dose
- Subjects will undergo a 12-lead ECG pre-dose
- Subjects will receive a single dose of the assigned IMP (Benfo-Oxythiamine (B-OT) within a time interval of two hours between 07:00 am and 09:00 am.
- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) and pharmacodynamic (PD) analysis according to the sample schedule in section 12.4.1 Blood samples collection
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.2.10 Procedures on Day 8

- Subjects will have their vital signs recorded
- Subjects will undergo a limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee)
- Subjects body weight will be measured
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, coagulation, urinalysis)
- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) and pharmacodynamic (PD) analysis according to the sample schedule in section 12.4.1 Blood samples collection
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will receive unified balanced lunch, afternoon snack and evening meal throughout the day

12.2.11 Procedures on follow-up Day 11

- Subjects will have their vital signs recorded
- Subjects will undergo limited physical examination to target any change from the previous status or any body system deemed pertinent to Subjects by the PI (or designee)
- Subjects body weight will be measured
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, coagulation, urinalysis)

- Catheter for blood sampling will be inserted and serial blood samples will be collected for pharmacokinetic (PK) analysis according to the sample schedule in section 12.4.1 Blood samples collection
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded

12.2.12 Procedures on End of study visit (Day 14)

- Subjects will come to the Clinical site for an ambulatory End of study visit
- Subjects will undergo a complete physical examination including documenting weight
- Subjects will provide nasopharyngeal and oropharyngeal swab which will be tested for the presence of SARS-COV-2 infection with a rapid antigen test
- Subjects will have their vital signs (heart rate, blood pressure, respiratory rate, body temperature) recorded
- Subjects body weight will be measured
- Subjects will provide blood and urine samples for laboratory analysis (biochemistry, hematology, coagulation, urinalysis); the urine sample will be used also for drug screen test and cotinine test performed via dip stick
- Subjects will undergo a 12-lead ECG
- Subjects will undergo a breath alcohol test
- Blood samples will be collected for PK analysis according to the sample schedule in section 12.4.1 Blood samples collection
- Adverse events (AEs) that have occurred will be recorded
- Concomitant medication intake will be recorded
- Subjects will complete the study and their participation will be ended

Every effort must be taken to ensure that subjects terminating the study prematurely undergo the safety checks of the follow-up visit. If subjects terminating the study prematurely due to an AE, every effort must be taken to monitor the subjects until the AE has resolved.

12.3 Safety and tolerability assessments

Only healthy subjects are eligible for the study. The healthy status will be determined by meeting the inclusion and exclusion criteria. Subjects' safety will be monitored during the study. Adverse events will be monitored and recorded throughout the study.

Prior to the first benfo-oxythiamine (B-OT) dosing only, the Principal Investigator or a delegated medical doctor will assess the clinical significance of results of laboratory investigations for hematology and serum chemistry values outside the defined normal ranges, as provided by the laboratory.

In addition to the planned times, any safety procedures can be performed at any time considered necessary by the Principal Investigator or a delegated medical doctor.

At least one qualified physician will be present at all times during each confinement day of each cohort. In addition, an ambulance and an emergency doctor will be available on each dosing day for a period of 12 hours (from 08:00 am until 08:00 pm) for the SAD part and for a period of 4 hours for the MAD part at the site and on call during the rest of the study. Also, two cardiologists will remain available on call at all times during the entire period of the study. In case any clinically significant abnormality is observed at pre-dose, Principal Investigator or

a delegated medical doctor will decide whether the subject will proceed to investigational product administration or will be withdrawn from the study.

Safety will be assessed by consideration of all adverse events reported by or elicited from the subject and abnormalities detected on physical examinations, safety blood tests, urinalysis, vital signs and 12-lead ECG.

All adverse events (serious and non-serious) beginning prior to dosing through follow-up visit will be recorded in the subject's source documentation and CRF.

The definitions, reporting and follow-up of AEs, SAEs and potential pregnancies are described in section 13.1.

12.3.1 Vital Signs

Evaluations of systolic and diastolic blood pressure, pulse rate, respiratory rate, and body temperature will be performed throughout the study. Pulse and blood pressure will be taken after 5 minutes in the supine position.

12.3.2 Weight and height

Weight (kg), height (cm) and body mass index (BMI) will be recorded/calculated at screening.

12.3.3 Physical examination

Physical examination (including general appearance, head, ears/eyes/nose/throat/neck, skin, respiratory system, cardiovascular system, gastrointestinal (including mouth) system, musculoskeletal system, central and peripheral nervous systems, and lymph nodes) will be performed by the Investigator(s) or a designee. Other body systems can be examined if required, at their discretion of the Investigator. Clinically relevant findings that are present prior to the first study drug administration must be recorded with the subject's Medical History. Clinically relevant findings found after study drug administration and meeting the definition of an AE (new AE or worsening of previously existing condition) must be recorded.

12.3.4 Electrocardiography

A complete standard 12-lead ECG will be recorded by a member of the investigator's team under the following conditions:

- Supine position of the subject for at least 5 minutes rest
- Use of a computerized ECG device
- Automatic calculation of the following parameters: heart rate, PR interval, QRS width, QT interval, and QTcF (calculated using Fredericia's formula)
- ECG printouts will be examined and interpreted by the investigator on the day of recording. This analysis performed by the investigator has to be entered in the eCRF. Any new clinically relevant abnormality that occurred after signing the informed consent will be documented as an AE.

The investigator will assess the ECG recording as 'normal', 'abnormal – not clinically significant', or 'abnormal – clinically significant' and include a description of the abnormality as required. ECG printouts will be collected and stored at the site. Each ECG trace should be labelled with the study number, subject number, subject initials and date of birth.

12.3.5 Laboratory assessments, drug screen and breath alcohol test

Routine laboratory safety samples will be analyzed by the local laboratory Bodimed ltd. The site will be supplied will kits for sample collection, requisition forms for sample shipment to analytical site and a laboratory manual with instructions for collection and handling of the

samples. The analysis of urine will be done at the site with urine dip stick tests, which will be purchased by the site team prior to the initiation of the study.

Printed laboratory reports will include normal reference ranges. A decision regarding whether the result outside the reference range is of clinical significance or not shall be made by an Investigator and the report will be annotated accordingly. Clinically significant abnormalities at screening or occurring during the study will be recorded on the AE page. The reference ranges for laboratory parameters will also be filed in the Investigator site file.

The laboratory results should be signed and dated and retained at clinical research site as source data for laboratory variables.

Any clinically significant deviation of laboratory parameters from reference ranges will be documented in the source documentation and in the eCRF and followed up, by the Principal Investigator or authorized investigator. Re-test of the safety laboratory parameters may be done under Investigator's discretion.

Blood and urine samples will be collected for the following safety clinical laboratory tests as listed in Table 3:

TABLE 3: SAFETY PARAMETERS

Laboratory Test/Safety Procedure	Parameters
SAD Part	
Hematology	Haemoglobin (HGB), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), hematocrit (HCT), red cell count (RBC), white blood cells (WBC) neutrophils (NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOS), basophils (BASO) and platelets (PLT).
Chemistry and electrolytes	Sodium, potassium, calcium, inorganic phosphate, total protein, albumin, glucose1, triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, total bilirubin2, alkaline phosphatase, AST, ALT gamma-GT and LDH.
Serology	HIV1 and HIV2 antibodies, Hepatitis B surface antigen, Hepatitis B antibodies and Hepatitis C antibodies
SARS-COV-2 (COVID – 19) infection	Polymerase Chain Reaction method for detection of the virus
Urinalysis (will be done using dip stick test at the site)	Leucocytes, blood, nitrite, protein, urobilinogen, bilirubin, pH, specific gravity, ketones, glucose.
Alcohol	Alcohol Breath Test
Urine drug screen	Cocaine, amphetamines, opiates, benzodiazepines and cannabinoids.
MAD Part	
Hematology	Haemoglobin (HGB), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), hematocrit (HCT), red cell count (RBC), white blood cells (WBC) neutrophils

	(NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOS), basophils (BASO) and platelets (PLT).
Coagulation	Fibrinogen, prothrombin time (PT), activated partial thromboplastin time (aPTT)
Chemistry and electrolytes	Sodium, potassium, calcium, inorganic phosphate, total protein, albumin, glucose1, triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, total bilirubin2, alkaline phosphatase, AST, ALT gamma-GT and LDH.
Serology	HIV1 and HIV2 antibodies, Hepatitis B surface antigen, Hepatitis B antibodies and Hepatitis C antibodies
SARS-COV-2 (COVID – 19) infection	Rapid antigen test for detection of the virus
Urinalysis (will be done using dip stick test at the site)	Leucocytes, blood, nitrite, protein, urobilinogen, bilirubin, pH, specific gravity, ketones, glucose.
Alcohol	Alcohol Breath Test
Urine drug screen	Cocaine, amphetamines, opiates, benzodiazepines and cannabinoids, barbiturates.

Urine drug test will be performed on site with a dip stick test and will include amphetamine, barbiturates, benzodiazepines, cocaine, cannabinoids, opioids and nicotine. At time-points when both urinalysis and drugs of abuse screening is required, all testing will be performed from a single sample.

An alcohol breath test will be conducted on site using a breathalyzer. The results of the test will be recorded in each subject's source documents and eCRF. Only subjects with a "0%" result will be considered to have a negative test result.

A full chain of custody will be maintained for all samples throughout their lifecycle.

The investigator will ensure full traceability of collected biological samples from the subjects while in storage at the center until shipment and will keep documentation of receipt of arrival.

The sample receiver will keep full traceability of samples while in storage and during use, until used, disposed of, or until further shipment or disposal (where appropriate) and will keep documentation of receipt of arrival.

12.3.6 Concomitant medications

In this study, limited types of concomitant medications are permitted. Adjustment of routine medications taken by subjects should be avoided during study participation except when subject safety could be affected by lack of adjustment

Concomitant medications initiated or stopped for an AE will be recorded in the source documentation and CRF of the relevant subject.

If a concomitant medication is used after the first treatment administration an investigator or delegate and/or the sponsor will decide whether the subject will be permitted to remain in the study, depending on the drug used, the time of drug intake, etc.

12.4 Pharmacokinetic and pharmacodynamics assessments

12.4.1 Blood samples collection

Blood samples for PK and PD determination will be processed, stored, and shipped according to the sample processing instructions supplied by the bioanalytical facility. All blood samples will be drawn using an indwelling IV catheter or by venipuncture. After blood sample collection from the IV catheter, the catheter will be flushed with a 0.9 % w/v NaCl solution to avoid obstruction (without Hep Lock). The indwelling IV catheter will not be left in situ for more than 24 hours, except during treatment period. If the intravenous catheter is not available for use and/or has been in place for > 24 hours, samples will be drawn by venipuncture, except during the treatment period.

SAD Study part:

PK blood samples (5 mL) for determination of plasma benfo-oxythiamine (B-OT), oxythiamine (OT) or other derived metabolites concentrations will be taken into pre-labelled EDTA collection tubes at the time points indicated in the Visit and Assessment Schedule (Table 1).

The quantitative determination of B-OT and OT in human plasma will be performed by using a validated method.

Samples should be maintained at 4 ºC (± 4 ºC) during the whole procedure from sample collection until sample freezing.

Samples will be cooled in an ice/water bath or cooling device until processed, centrifuged at approximately 1900 g (± 38g) at 4°C (± 4°C) for 10 minutes and then placed into an ice/water bath or cooling device. The time between blood collection and placement in the centrifuge will not exceed 60 minutes.

Plasma will be divided and transferred into pre-labeled duplicate 2 mL polypropylene tubes for each analyte (B-OT and OT). The resulting 4 aliquots will be maintained in the ice/water bath or cooling device. A minimum of 0.5 mL of plasma is required for each aliquot.

Samples will be kept in an ice/water bath until transferred to the storage unit (freezer).

Then, samples will be stored and frozen at approximately -80ºC (±15ºC) until shipment to the bioanalytical laboratory. The time between the centrifuge and placement in freezer will not exceed 60 minutes.

MAD Study part:

PK blood samples (2.0 mL) for determination of plasma oxythiamine (OT) and any other derived metabolites concentrations will be taken into pre-labelled EDTA collection tubes at the time points indicated in the Visit and Assessment Schedule (Table 2).

The quantitative determination of OT and any other derived metabolites in human plasma will be performed by using a validated method.

Samples should be maintained at 4 ºC (± 4 ºC) during the whole procedure from sample collection until sample freezing.

Samples will be cooled in an ice/water bath or cooling device until processed, centrifuged at approximately 1900 g (± 38g) at 4°C (± 4°C) for 10 minutes and then placed into an ice/water bath or cooling device. The time between blood collection and placement in the centrifuge will not exceed 60 minutes.

Plasma will be divided and transferred into pre-labeled duplicate 2 mL polypropylene tubes The resulting 2 aliquots will be maintained in the ice/water bath or cooling device. A minimum of 0.5 mL of plasma is required for each aliquot.

Samples will be kept in an ice/water bath until transferred to the storage unit (freezer).

Then, samples will be stored and frozen at approximately -80ºC (±15ºC) until shipment to the bioanalytical laboratory. The time between the centrifuge and placement in freezer will not exceed 60 minutes.

In the SAD part, PD blood samples (2 mL) for transketolase activity analysis will be managed according to the instructions leaflet dated 08 Nov 2021, provided by the responsible laboratory Medical Laboratory Bremen and are the following:

a. Collect samples in pre-labelled EDTA (2 mL) collection tubes at the time points indicated in the Visit and Assessment Schedule (Table 1).
b. Transfer the sample material into a reagent tube of approx. 13 ml capacity, preferably made of polypropylene, e.g. SARSTED, 95 mm x 16.8 mm, article no. 55.518. Polystyrene tubes are very fragile when frozen (see below).
c. Fill up the tube with isotonic (0.9%) NaCl solution.
d. Centrifugation is then performed at room temperature in a standard laboratory centrifuge at approx. 2800 g, which corresponds to 3800 rpm when e.g. SIGMA 3-16P K centrifuge is used, centrifugation time is 10 min, brake acceptable.
e. Remove the supernatant (including the buffy coat) and fill up again with isotonic NaCl solution.
f. Then centrifuge again, when removing the supernatant, immerse about 1 mm deep into the erythrocyte sediment, fill up with isotonic NaCl.
g. Repeat the above steps again, remove the supernatant for the last time. Mix 0.5 ml of the washed and packed erythrocytes with 0.5 ml of distilled water (not isotonic NaCl!).
h. Mix thoroughly (e.g. VORTEX mixer 10 sec), by this step most of the erythrocytes are hemolyzed and their components are released.
i. Freeze at -70°C until shipment to the laboratory within the planned schedule of shipments

In the MAD part, PD blood samples (5 ml) will be collected in prelabelled EDTA collection tubes at time points indicated in the visit and assessment schedule (Table 2). The samples will be prepared and analyzed according to a validated method if available.

The following blood samples (Table 4) need to be drawn for PK and PD analysis on each of the sampling days, as indicated in the Visit and Assessment Schedule (Table 1 and 2).

TABLE 4: PK/PD SAMPLING TIMEPOINTS

SAD part			
PK sample timepoint (hr.)	**Allowed time deviation**	**PD sample timepoint (hr.)**	**Allowed time deviation**
Pre-dose (35 min before dosing)	+/- 5 min	Pre-dose (35 min before dosing)	+/- 5 min
0.25	+/- 5 min	-	-
0.5	+/- 5 min	-	-
0.75	+/- 5 min	-	-
1.0	+/- 5 min	1.0	+/- 5 min
1.5	+/- 5 min	-	-
2	+/- 5 min	2.0	+/- 5 min
4	+/- 10 min	4.0	+/- 10 min
8	+/-15 min	8.0	+/-15 min
12	+/- 30 min	12	+/- 30 min
24	+/- 1 hr	24	+/- 1hr
36	+/- 1 hr	-	-
48	+/- 1hr	48	+/- 1hr
72	+/- 1hr	72	+/- 1hr
168	+/- 2 hr	168	+/- 2 hr

MAD part				
Day	**PK sample timepoint (hr.)**	**Allowed time deviation**	**PD sample timepoint (hr.)**	**Allowed time deviation**
1	Pre-dose (35 min before dosing)	+/- 5 min	Pre-dose (35 min before dosing)	+/- 5 min
	0.25	+/- 5 min	-	-
	0.5	+/- 5 min	-	-
	0.75	+/- 5 min	-	-
	1.0	+/- 5 min	1.0	+/- 5 min
	1.5	+/- 5 min	-	-
	2	+/- 5 min	2.0	+/- 5 min
	4	+/- 10 min	4.0	+/- 10 min
	8	+/-15 min	8.0	+/-15 min
	12	+/- 30 min	12	+/- 30 min
2	Pre-dose (35 min before dosing; 24 hr after last dosing	+/- 30 min	Pre-dose (35 min before dosing; 24 hr after last dosing	+/- 30min
3	Pre-dose (35 min before dosing; 24 hr after last dosing	+/- 30 min	-	
4	Pre-dose (35 min before dosing; 24 hr after last dosing	+/- 30 min	Pre-dose (35 min before dosing) - - - 1.0 - 2.0 4.0 8.0 12	+/- 30 min
5	Pre-dose (35 min before dosing; 24 hr after last dosing	+/- 30 min	Pre-dose (35 min before dosing; 24 hr after last dosing	
6	Pre-dose (35 min before dosing; 24 hr after last dosing	+/- 30 min	-	+/- 30 min
7	Pre-dose (35 min before dosing)	+/- 5 min	Pre-dose (35 min before dosing)	+/- 5 min
	0.25	+/- 5 min	-	-
	0.5	+/- 5 min	-	-
	0.75	+/- 5 min	-	-
	1.0	+/- 5 min	1.0	+/- 5 min
	1.5	+/- 5 min	-	-
	2	+/- 5 min	2.0	+/- 5 min

MAD part				
Day	**PK sample timepoint (hr.)**	**Allowed time deviation**	**PD sample timepoint (hr.)**	**Allowed time deviation**
	4	+/- 10 min	4.0	+/- 10 min
	8	+/-15 min	8.0	+/-15 min
	12	+/- 30 min	12	+/- 30 min
8	24 hr after last dosing	+/- 30 min	24 hr after last dosing	+/- 30 min
11	96 hr after last dosing	+/- 4hr	-	-
14	168 hr after last dosing	+/- 4hr	-	-

The clock time will be recorded and reported for all blood draws and all subjects. Actual sampling time will be used for pharmacokinetic calculations. A deviation greater than the one indicated as allowed will be reported as a protocol deviation in a master protocol deviation log and its cause will be recorded.

When the following assessments are scheduled to be performed at the same time-point, the order of priority will be as follows:

1. ECG
2. Vital signs
3. Blood sampling for safety
4. Physical examination

When a PK assessment is scheduled for the same nominal time as another scheduled assessment, the PK sample will have priority, unless other procedures are necessary for assuring subject's safety.

If an assessment or collection is scheduled by day, and not by a specific time, then no time window will apply although generally the time of day should be the same throughout the study.

12.4.2 Labelling

Pre-printed, waterproof labels will be used to identify all PK and PD tubes used during blood sample collection and for storage of separated plasma. Each label will contain the following information:

- Protocol number
- Subject Number
- Study period number (e.g. D-1)
- Blood Sample Number
- Sampling time point (e.g. 0:30m; 1:00h)
- Sample type (blood or plasma) & purpose (e.g. PK)

12.4.3 Shipping procedures

The primary PK and PD samples will be stored at the clinical facility until the shipment to the respective analytical Biochemical Laboratories The back-up samples can be shipped to laboratory only if required, after Sponsor written approval.

The CRO will arrange shipment of the PK and PD samples. Samples must be shipped to the Analytical Biochemical Laboratories at time intervals agreed with the Sponsor. The samples

will be transferred in an appropriate container packed in an adequate amount of dry ice enough to keep the samples frozen for at least 72 hours. For all shipments, the laboratory should acknowledge in writing the receipt of plasma samples in good condition.

The samples destined for retention will be stored by the clinical facility in a freezer at -80°C and will remain there until the final report is issued and the study sponsor authorization is obtained to discard the samples. The packaging of the samples will meet the Biosafety rules and IATA recommendations related to the documentation and package.

12.4.4 Determination of plasma drug concentrations and measurement of the transketolase activity

The concentration in Plasma of B-OT, OT and potential other Thiamine metabolites derivate will be determined using a validated liquid chromatography tandem mass spectrometry method and will be conducted in accordance with Good Laboratory Practice regulations and guidelines. Methods validation must be completed prior to start of the analysis.

In the SAD part, For each cohort, in preparation of the SRC, the PK analysis is conducted after all Day 2 (T=48hr) are taken. The rest of the PK samples ((T=72 and 168hr) are analysed after all PK samples are collected at the end of the study. In each run, standard and quality control samples will be distributed throughout the batch containing study samples. Samples with concentrations above the ULOQ will be diluted and re-assayed.

In the MAD part, for each cohort, in preparation of the SRC, the PK analysis will be conducted after all Day 5 samples are taken. The rest of the PK samples are analysed after all PK samples are collected at the end of the study. In each run, standard and quality control samples will be distributed throughout the batch containing study samples. Samples with concentrations above the ULOQ will be diluted and re-assayed. As B-OT was not detected in any cohort of the SAD part, the concentration of B-OT will not be assessed in the MAD. Other metabolites (e.g oxythiamine pyrophosphate) of this prodrug might however be assessed from the back up samples if a validated method has been established before the end of the study.

Full details, including the lower (LLOQ) and upper (ULOQ) limits of quantification range, will be provided in a Bioanalytical Plan

Plasma samples of withdrawn subjects should also be analyzed for drug concentrations and pharmacokinetic parameters (providing withdrawal was not due to withdrawal of consent).

Unacceptable values due to analytical reasons will be handled in accordance with the Bioanalytical Plan.

Measurement of the transketolase activity in erythrocytes will be done following the routinely established procedure to perform the analysis in Medical Laboratory Bremen in the SAD part of the study.

In the MAD part of the study transketolase activity measurement will be done according to a validated method established by the responsible laboratory if available.

12.2.5 Total blood volume and appropriateness of measurements

All measurements performed in the study are standard measurements.

The total volume of blood to be collected from each subject during the study as listed in table 5 (approximately 139 mL) is considered acceptable.

TABLE 5: TOTAL VOLUME OF BLOOD PER SUBJECT

Sample	Number of samples	Sample volume	Total volume
SAD part			
Hematology	5	3 mL	15 mL
Chemistry	5	4.5 mL	22.5 mL
Serology	1	6 mL	6 mL
PK	15	5 mL	75 mL
PD (TKT activity)	10	2 mL	20 mL
Total blood volume/subject (SAD)			138.5 mL
MAD part			
Hematology	7	3 mL	21 mL
Chemistry	7	5 mL	35 mL
Coagulation	7	2.7 mL	19 ml
Serology	1	6 mL	6 mL
PK	28	2 mL	56 mL
PD (TKT activity)	21	5 mL	105mL
Total blood volume/subject (MAD)			242 mL

13 SAFETY REPORTING

13.1 Definitions of adverse events

The definitions are adopted in accordance with the Directive 2001/20/EC, ICH-E2A and the European Commission Detailed Guidance 2011/C 172/01.

An **Adverse Event (AE)** is defined as any untoward medical occurrence in a clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with the treatment.

An AE can therefore be any unfavourable and unintended sign (including any clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

The following **should not be recorded as AEs**, if recorded as medical history/concomitant illness at screening:

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject has signed the informed consent
- Pre-existing conditions found as a result of screening procedures.

A clinically significant deterioration of a value for a laboratory parameter should be recorded as an adverse event. For alterations to clinical laboratory parameters over the course of the study, a "significant deterioration in laboratory parameters" indicates an abnormal laboratory result, which is deemed clinically significant when any of the following conditions are met:

- The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline.
- The abnormality is of a degree that requires additional active management, e.g., change of treatment, discontinuation of treatment, close observation, more frequent follow-up assessments, or further diagnostic investigation.

An **Adverse Drug Reaction (ADR)** means all untoward and unintended responses to an investigational medicinal product related to any dose administered. The phrase 'response to a medicinal product' means that a causal relationship has at least a reasonable possibility, i.e. the relationship cannot be ruled out and is judged by the Investigator as at least possible (see definition below).

An **Unexpected Adverse Drug Reaction (UADR)/Unexpected Adverse Event (UAE)** means an adverse reaction/event, the nature or severity of which is not consistent with the applicable product information, namely in the Investigator Brochure for an unauthorized investigational product or in the SmPC for an authorized product.

The expected/unexpected status should be evaluated and assessed, by the Sponsor, based on the reference safety information available since expectedness in Pharmacovigilance refers strictly to the information listed or mentioned in the applicable reference safety information and not to events that might be anticipated from knowledge of the pharmacological properties of a substance or because it was foreseeable due to the health status (e.g., age, medical history) of the study subjects.

A **Serious Adverse Event (SAE)** or **Serious Adverse Reaction (SAR)** is defined as an AE that results in any of the following:

- Results in death

- Is life-threatening
- Requires hospitalization or prolongs existing inpatient's hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital abnormality or birth defect
- Is an important medical event which requires medical intervention to prevent any of the above outcomes

SUSARs: AEs which meet all of the following criteria:

- Serious.
- Unexpected (i.e., is not consistent with the applicable product information e.g. Investigator's brochure for an unapproved benfo-oxythiamine (B-OT) or SmPC for an authorised product).
- Suspected (i.e., there is at least a reasonable possibility that there is a causal relationship between the event and the medicinal product).

will be classified as suspected unexpected serious adverse reactions (SUSARs).

Important medical events are those which may not be immediately life-threatening, but may jeopardize the subject and may require intervention to prevent one of the other serious outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, or blood dyscrasias or convulsions that do not result in hospitalization.

The term "**life-threatening**" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. For example, drug-induced hepatitis that resolves without evidence of hepatic failure would not be considered life threatening even though drug-induced hepatitis can be fatal.

Inpatient hospitalization or prolongation of existing hospitalization means that hospital inpatient admission and/or prolongation of hospital stay were required for treatment of AE or occurred as a consequence of the event. It does not refer to pre-planned elective hospital admission for treatment of a pre-existing condition that has not significantly worsened, or to diagnostic procedures.

13.1.1 Severity of adverse events

The intensity of clinical AEs is graded three-point scale as defined below:

- Mild: discomfort noticed but no disruption of normal daily activity;
- Moderate: discomfort sufficient to reduce or affect normal daily activity, requiring no or minimal medical intervention;
- Severe: inability to work or perform daily activity, requires medical intervention

13.1.2 Relationship to study drug

For each AE the relationship to the study medication as judged by the investigator will be as follows:

Category	Description
Not Related	A clinical event, including laboratory test abnormality that has no temporal relationship to the study medication or has more likely alternative etiology.

Unlikely	A clinical event, including laboratory test abnormality, with little or no temporal relationship to study medication administration, and which other drugs, chemicals or underlying disease provide plausible explanations.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the study medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Probable	A clinical event, including laboratory test abnormality, which follows a reasonable temporal sequence from the time of study medication administration, and/or follows a known response pattern to the product, and could not have been produced by other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy.
Certain	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the study medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.

13.1.3 Chronicity of adverse events

The chronicity of the AE will be classified by the investigator on a three-item scale as defined below:

- Single occasion: single event with limited duration;
- Intermittent: several episodes of an event, each of limited duration;
- Persistent: event which remained indefinitely

13.1.4 Action

The Investigator must provide the action taken with regard to each AE as:

- None
- Study drug dosing interrupted
- Use of intervention to treat the AE (provide details, recording concomitant therapy, if needed)
- Discontinuation of the study drug

13.1.5 Outcome of adverse event

The Investigator must provide the outcome of the adverse event as:

- Recovered/Resolved
- Recovered/Resolved with sequelae
- Recovering/Resolving
- Not recovered/Not resolved
- Fatal
- Unknown

Sequelae: The signs/symptoms of the reported AE have not completely resolved and a new baseline for the subject is established since full recovery is not expected.

13.1.6 Reporting of adverse events and serious adverse events

Time period for collection of adverse events and serious adverse events:

Adverse events will be recorded from the time of informed consent up to and including the follow-up visit.

AEs which occurred prior to initiation of study medication will be recorded as screening events or as part of the medical history (as applicable), only occurrences related to protocol mandated procedures or ongoing at first dosing should be reported as AE.

AEs occurring after initiation of study treatment will be indicated as TEAEs in the clinical study report.

The following variables will be collected for each AE:

- Adverse event (verbatim)
- The date and time when the AE started and stopped
- Maximum intensity
- Whether the AE is serious or not
- Investigator causality rating against the IMP (acc. to categories as specified in 13.1.2)
- AE caused healthy volunteer's withdrawal from study (yes or no)
- Outcome

Reporting of Serious Adverse Events

All SAEs have to be reported, whether or not considered causally related to the IMP, or to the study procedure(s). All SAEs will be recorded in a SAE report form (Annex No 1) and reviewed and evaluated by a Sponsor's representative. The eCRF will contain information about all adverse events that were evaluated as "serious".

If any SAE occurs in the course of the study, then Investigators or other site personnel must inform the designated safety contact people on behalf of the Sponsor and CRO within 24 hours of when he or she becomes aware of its occurrence.

SAE report form completion and reporting must not be delayed even if all of the information is not available at the time of the initial contact.

SAEs must be reported within 24 h of knowledge of the event by submitting an initial SAE report via email or fax to the safety representatives. All details will be provided in the safety management plan.

The following information should be provided to accurately and completely record the event:

- Investigator name and center identification
- Subject number
- Subject initials, if permitted
- Subject demographics
- Clinical event
  - description
  - date of onset
  - severity
  - treatment
  - relationship to study drug (causality)
  - action taken regarding study drug

- outcome of the event (recovered/resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with sequel, fatal, unknown)

- If the AE resulted in death:
  - cause of death (whether or not the death was related to study drug)
  - autopsy findings (if available)
- Medical history case report form (copy)
- Concomitant medication case report form (copy)
- Any relevant reports (laboratory, discharge, x-ray, etc.)

The Sponsor representative will work with the Investigator to compile all the necessary and initially missing information within 1 calendar day of initial receipt for fatal and life-threatening events and within 3 calendar days of initial receipt for all other SAEs.

Unexpected (not previously described in the reference safety document), Serious Adverse Reactions (SUSARs) will be expedited by the Sponsor representative to Eudravigilance using CIOMS (Council for International Organisations of Medical Sciences) form.

all details will be provided in the Safety Management Plan

13.1.7 Follow-up of adverse events

All AEs will be followed until they have abated, returned to baseline status or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

13.2 Temporary halt for reasons of subject safety

The investigator will inform the subjects and the EC 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. The study will be suspended pending further review by the EC, except insofar as suspension would jeopardise the subjects' health. The investigator will ensure that all subjects are kept informed.

14 STATISTICAL METHODOLOGY AND ANALYSES

14.1 Statistical analysis plan

The study is exploratory and is not powered to address any pre-defined hypotheses. The assessment of safety and tolerability will be performed on the safety analysis set, which includes all subjects who received at least one dose of B-OT. Complete description of all statistical analyses and methods will be presented in the Statistical Analysis Plan (SAP). The SAP will be reviewed and approved by the Sponsor and will be finalized prior to database lock. Plans for PK analyses will be included in the SAP.

The sample sizes for the SAD cohorts are typical for a Phase 1 first in human (FIH) study. The total number of subjects accrued in the study will depend on the number of dose levels, but is currently set to a maximum of 24 subjects (2 cohorts with 3 subjects and 3 cohorts with 6 subjects each). The safety and tolerability of B-OT will be analyzed by appropriate descriptive statistics. All other secondary endpoints will be summarized descriptively.

The sample sizes for the MAD cohorts are typical for a Phase 1 multiple ascending dose study. The total number of subjects in the study will be 24 subjects (4 cohorts with 6 subjects each). The safety and tolerability of B-OT will be analyzed by appropriate descriptive statistics. All other secondary endpoints will be summarized descriptively.

If circumstances should arise during the study rendering the analysis inappropriate, or if in the meantime improved methods of analysis should come to light, different analyses may be performed. Any deviations from the statistical methodology defined in this protocol, reasons for such deviations and all alternative/additional statistical analyses that may be performed will be described in the CSR. Such changes to analyses may be written into an abbreviated SAP, if appropriate. The verification and review of all statistical Modelling assumptions will be documented appropriately.

All original and derived parameters as well as demographic and disposition data will be listed and described using summary statistics. All safety data (scheduled and unscheduled) will be presented in the data listings.

Demographic and baseline data will be summarised by treatment (dose level of B-OT) and overall. Pharmacokinetic data will be summarised by dose level of B-OT. Safety and tolerability data will be summarised.

Frequency counts (number of subjects [n] and percentages) will be made for each qualitative variable. Descriptive statistics (n, mean, SD, min, median, max) will be calculated for each quantitative variable (unless otherwise stated). Descriptive statistics will only be presented if $n \geq 3$. If no subjects have data at a given time point, then only n=0 will be presented. If $n < 3$, only the n, minimum and maximum will be presented. The other descriptive statistics will be left blank.

The following rules will apply to any repeated safety assessments occurring within each cohort:

- If the repeated measurement of a specific parameter occurs prior to IMP administration (Day 1), then the last obtained value prior to dosing will be used in the descriptive statistics and in the calculation of changes from baseline.
- If the repeated measurement of a specific parameter occurs after IMP administration (Day 1), then the first (non-missing) value after dosing will be used in descriptive statistics and in the calculation of changes from baseline.

The planned sequence for measurement of multiple assessments at the same time point is described in Section 12.2.1 Blood samples collection.

For safety assessments performed at Screening and the follow-up, the following rules will apply for any repeated assessments:

- If the repeated assessment occurs at Screening the last available value will be used in the summary statistics.
- If the repeated assessment occurs at the Follow-up Visit the first non-missing assessment will be used in the summary statistics. All statistical analyses and production of tables, figures and listings will be performed using current SAS® version (9.4), whereas the pharmacokinetic analysis will be done using WinNonlin® (version 8.3), or higher.

Details will be given in a statistical analysis plan (SAP) which will be prepared and validated by the Sponsor before database lock. Modifications or additions to the analyses described above will be described in the SAP. Any decisions to deviate from the planned analyses described in the protocol and SAP will be documented in the clinical study report. The clinical study report will also provide a detailed explanation for any deviations from the planned analyses.

14.2 Protocol violations/deviations

Protocol deviations will be identified based on conditions related to the categories below:

- Protocol entry criteria.
- Forbidden concomitant medications.
- Missing evaluations for relevant endpoints.
- Other protocol deviations occurring during study conduct.

Major protocol deviations will be identified before the study closure and listed where appropriate.

14.3 Power calculation

Not applicable.

14.4 Missing, unused and spurious data

All missing or incomplete safety data, including dates and times, are treated as such. Missing test results or assessments will not be imputed. Descriptive statistics and statistical analysis will be performed on the basis of the available data only.

Subjects discontinued will be included in the descriptive statistics if they have received the investigational product and their examinations were performed at the same scheduled time as other subjects. All data recorded on discontinued subjects will be listed separately.

For graphical and summary purposes concentration values below the limit of quantification will be set to half (½) of the limit of quantification. For analysis no undetermined values will be replaced. If single data points for plasma B-OT or OT concentrations are missing, the AUC parameters will be derived by linear interpolation with regard to the two neighboring non-missing concentrations.

For calculation of PK parameters, all plasma B-OT and OT concentration values below the Lower Limit of Quantitation (LLOQ) occurring prior to Cmax will be replaced by 0(of Day 1), except for embedded LLOQ values (between two measurable time points) which will be treated as "missing". All below LLOQ values after Cmax will be treated as "missing" except at Day 7 pre-dose, where it will be replaced by LLOQ/2. The handling of missing, unused and spurious data will be documented in the study report.

14.5 Analysis sets

Data of all subjects participating in the study will be included in the analyses if the data can meaningfully contribute to the objectives of the study. Subjects who do not complete the sampling schedule of 1 or more study periods may be included in the PK population for only the PK parameters that are judged not to be affected by the missing sample(s).

The decision of which subjects will be included in the PK Analysis Set is to be taken before the start of the sample analysis by the bioanalytical facility.

14.5.1 Safety set

The safety population will be defined as all subjects who received at least one dose of B-OT and for whom any safety post-dose data are available.

Unless otherwise stated, the safety analysis set will be used for the presentation of all safety analyses. Exposure to IMP will also be presented using the safety analysis set.

14.5.2 Pharmacokinetic and pharmacodynamic analysis set

Subjects will be allocated to the PK Analysis Set on a per treatment basis. The PK Analysis Set for a treatment will include all subjects who receive one dose of B-OT, do not violate the protocol in a way that may invalidate or bias the results (major protocol deviation) and have sufficient plasma concentration-time profiles complying with the following criteria at Day 1 (SAD/MAD) and at Day 7 (MAD only):

- Do not have an occurrence of vomiting or diarrhoea which renders the plasma concentration profile unreliable (e.g., if vomiting occurs at or before 2 times median Tmax);
- Do not use a concomitant medication which renders the plasma concentration profile unreliable;
- Do not have a pre-dose plasma concentration that is greater than 5% of the Cmax value. If the predose concentration is greater than 5% of the Cmax value, the subject will be excluded from the PK population;
- Have at least one evaluable plasma concentration that is preceded by a lower evaluable concentration and followed by a lower evaluable concentration for the calculation of Cmax, Tmax and AUCs.

In case of an important protocol deviation or AEs, affected PK data will be excluded from the descriptive and inferential statistical. Where all PK data for a subject are impacted, the subject will be excluded from the PK analysis set. The exclusion of any subjects or time points from the calculation of the PK parameters will be documented by the statistician including the reason(s) for exclusion.

The PD analysis set will consist of all subjects in the safety analysis set who received at least one dose of B-OT and who have at least one post-dose values present, with no important

protocol deviations thought to impact on the analysis of the PD data. .PD analysis is an exploratory end point

14.6 Subject disposition

Subject disposition will be listed by subject.
The following subject data will be summarized:

- Number and percentage of subjects screened,
- Number and percentage of subjects enrolled,
- Number and percentage of subjects completed and,
- Number and percentage of subjects included in safety population,
- Number and percentage of subjects used in PK, PD and statistical analyses.

A subject who completed the study is defined as a subject for whom the last PK blood sample was assessed.

14.7 Baseline parameters

Baseline is defined as the last value prior to dosing. Change from baseline will be calculated for all continuous safety parameters.

14.7.1 Demographics and baseline variables

Continuous demographic variables (e.g., age, height, weight, BMI) will be summarized by descriptive statistics (n, mean, SD, median, Min, Max).
Qualitative demographic characteristics (sex, race/ethnicity) will be summarized by counts and percentages.

14.7.2 Medical history

Medical history will only be listed. Medical history data will be listed by subject including visit, description of the disease/procedure, MedDRA SOC, MedDRA Preferred Term, start date and stop date (or ongoing if applicable).

A summary of the number and percentage of subjects who had relevant medical histories will be presented by treatment group and for all subjects for the medical history Preferred Term.

14.8 Safety and tolerability endpoints

The safety set is used to perform all safety analyses. Statistical methods for the safety analyses will be descriptive in nature. Safety data, including AEs, clinical laboratory data, vital signs, ECG parameters, and PEs. All appropriate AEs will be graded using the DMID toxicity scale (March 2014). Change from baseline will be included in summary tables for laboratory parameters. All laboratory data will be included in the data listings and all test values outside the normal range will be flagged.

14.8.1 Adverse events

The AE coding dictionary for this study will be Medical Dictionary for Regulatory Activities (MedDRA). It will be used to summarize AEs by primary system organ class (SOC) and preferred term (PT). All adverse events will be displayed in listings.

A treatment-emergent adverse event (TEAE) is defined as an adverse event observed after starting administration of study treatment. If a subject experiences an event both prior to and after starting administration of a treatment, the event will be considered a TEAE (of the treatment) only if it has worsened in severity (i.e., it is reported with a new start date) after starting administration of the specific treatment, and prior to the start of another treatment, if any. All TEAEs collected during the investigational period will be summarized.
The number of subjects with treatment emergent AEs will be summarized by:

- Treatment, MedDRA SOC and PT;
- Treatment, MedDRA SOC, PT and severity;
- Treatment, MedDRA SOC, PT and drug relatedness.

14.8.2 Vital signs

At each time point, absolute values and change from baseline of supine blood pressure, pulse rate and respiratory rate will be summarized with n, mean, SD, SEM, median, Min, and Max values. The number of available observations and out-of-range values (absolute and in percentage) will be presented. Values outside the reference range will be flagged in the listing. 'H' and 'L', denoting values above or below the investigator reference range (when present), will flag out-of-range results.

14.8.3 ECG

At each time point, absolute values and change from baseline of ECG numeric variables will be summarized with n, mean, SD, SEM, median, Min, and Max values. The number of available observations and out-of-range values (absolute and in percentage) will be presented. Values outside the investigator's normal range will be flagged in the listing. 'H' and 'L', denoting values above or below the investigator reference range (when present), will flag out-of-range results.

14.8.4 Clinical laboratory tests

At each time point, absolute values and change from baseline of clinical laboratory variables will be summarized with n, mean, SD, SEM, median, Min, and Max values. The number of available observations and out-of-range values (absolute and in percentage) will be presented. All laboratory data (including re-check values if present) will be listed chronologically. Out-of-range values for safety laboratory will be flagged in individual listings as well as summarized descriptively using agreed reference ranges (e.g., laboratory ranges).

14.9 Pharmacokinetic and pharmacodynamic endpoints analysis

SAD study part:
Plasma PK parameters for each dose level will be calculated from the concentrations of B-OT and OT measured in pre-dose and post-dose plasma samples. For each dose level, descriptive statistics will be presented. Figures will be created to display mean and individual subject B-OT and OT concentration versus time.

MAD study part:
Plasma PK parameters for each dose level will be calculated from the concentrations OT measured in pre-dose and post-dose plasma samples. For each dose level, descriptive statistics will be presented. Figures will be created to display mean and individual subject OT concentration versus time.

The plasma concentrations and the PK parameters will be listed and presented in tabular and graphical form as appropriate according to the applicable standards, that includes applicable descriptive statistics, handling of individual concentrations below the LLOQ for listings, descriptive statistics and figures, and precision and rounding rules for concentrations and PK parameter data.
A 72-hour individual baseline profile of plasma B-OT and OT concentrations will be measured. Values will be plotted and descriptive statistics per planned sampling time point and treatment will be provided, including mean, SD, CV%, minimum, maximum, and median as well as geometric mean and its CV%.

Individual plasma B-OT and OT concentrations will be plotted versus time per individual using both a linear and log y-axis. Additionally, concentration versus time curves will be plotted per as a spaghetti plot. Individual subject listings will also be provided. Concentrations will be summarized for each time point of measurement, the number of observations, mean, SD, CV%, median, Min, and Max, as well as geometric means and its CV%. 95% confidence intervals will be given.

At least the following individual PK parameters will be determined based on the concentration versus time curves in the SAD part:

- maximum concentration (Cmax),
- time to Cmax (Tmax),
- elimination rate constant (λ_z),
- terminal elimination half-life (t1/2),
- area under the concentration versus time curve (AUC) from 0 to the last measurement point (AUC0-last)
- AUC extrapolated to infinity (AUC0-∞),
- Clearance (CL/F)
- Apparent volume of distribution (Vz/F)

A 168-hour individual profile of plasma OT concentrations and potentially other derivate metabolites will be measured. Values will be plotted and descriptive statistics per planned sampling time point and treatment will be provided, including mean, SD, CV%, minimum, maximum, and median as well as geometric mean and its CV%.

At least the following individual PK parameters will be determined based on the concentration versus time curves in the MAD part on Day 1: AUC_{τ}, C_{max}, t_{max}, on Day 7: AUC_{τ}, $C_{max,ss}$, $t_{max,ss}$, Swing, PTF%, CL_{ss}/F, V_z/F, C_{trough}, C_{av}, C_{min}, $t_{1/2}$, AUC_{inf} and AUC_{last} as appropriate and Day7/Day1: Ra(AUC_{τ}), Ra(C_{max}).

The lin-up log-down trapezoidal method will be used. If the logarithmic trapezoidal rule fails in an interval because non-monotonicity, then the linear trapezoidal rule will apply for that interval.

If the terminal phase is sufficiently well characterized the terminal half-life will be estimated and AUC zero to infinity (AUC0–∞) will be derived from AUC0-last and the extrapolated area from the last measurement point to infinity based on the terminal half-life. Within a subject, data points before time of maximal plasma concentration that are below the lower limit of quantification (LLOQ) will be replaced with zero. Within a subject, data points after time of

maximal plasma concentration that are below the LLOQ will be excluded from analysis (considered as missing). Additionally, Cmax and tmax will be determined.

The individual PK parameters will be summarized, including at least number of subjects, mean, SD, CV%, median, Min, and Max, as well as geometric means and its CV%.

14.9.1 Dose proportionality

Dose-proportionality for PK parameters of OT and B-OT (AUC0-inf, AUClast, and Cmax for single dose, and $AUC_{0-\tau\ Day7}$, and $C_{max,ss}$ for multiple dose) will be assessed by the power model. The slope and the associated 90% CI based on the power model will be reported. For the first 2 low doses of the SAD part where only 3 subjects are enrolled, no dose proportionality will be assessed.

14.9.2 Inferential methods

Not applicable.

14.10 Interim analyses

No formal interim analyses are planned for this study. Data from SAD cohorts were frozen and analyzed prior to conducting the s MAD part of the study.

15 GOOD CLINICAL PRACTICE, ETHICS AND ADMINISTRATIVE PROCEDURES

15.1 Good clinical practice

15.1.1 Ethics and good clinical practice

The investigator will ensure that this study is conducted in full compliance with the protocol, the principles of the Declaration of Helsinki (www.wma.net), ICH GCP guidelines (http://www.ich.org/products/guidelines.html), and with the laws and regulations of the country in which the clinical research is conducted.

15.1.2 Ethics committee / institutional review board

The CRO on behalf of the investigator will submit this protocol and any related documents to an Ethics Committee (EC) and the Competent Authority (CA). Approval from the EC and the statement of no objection from the CA must be obtained before starting the study (i.e. first subject first visit), and should be documented in a dated letter to the applicant, clearly identifying the trial, the documents reviewed and the date of approval. A list of EC members must be provided, including the functions of these members. If study staff were present, it must be clear that none of these persons voted.
Modifications made to the protocol after receipt of the EC and CA approval must also be submitted as amendments to the EC and CA in accordance with local procedures and regulations.

15.1.3 Informed consent

The Informed Consent and Subject Information will be provided in the local language – Bulgarian.
The Principal Investigator or delegate will:

- Ensure each healthy volunteer is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each healthy volunteer is notified that they are free to discontinue from the study at any time
- Ensure that each healthy volunteer is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each healthy volunteer provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed ICF(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed ICF is given to the healthy volunteer
- Ensure that any incentives for healthy volunteers who participate in the study as well as any provisions for healthy volunteers harmed as a consequence of study participation are described in the ICF that is approved by an EC.
- Ensure subjects are re-consented to the most current version of the ICF(s) during their participation in the study.

A subject who is rescreened is not required to sign another ICF if the rescreening occurs within 28 days from the previous ICF signature date.

15.1.4 Insurance

The sponsor has covered this clinical study by means of an insurance according to national requirements in Bulgaria. This insurance will cover the sponsor´s and the investigator´s

liability. The name and address of the relevant insurance company, the certificate of insurance, the policy number and the sum insured are provided in the Investigator's Site File.

15.2 Study funding

benfovir AG is the sponsor of the study and is funding the study. All financial details are provided in a separate contract(s) between Convex and the sponsor.

15.3 Data handling and record keeping

15.3.1 Data collection and confidentiality

A Subject Screening and Enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.
The data collection tool for this study will be an electronic CRF. For each subject enrolled, regardless of the occurrence of study drug administration, eCRF must be completed and signed by the principal investigator or co-investigator. This also applies to those subjects who fail to complete the study. If a subject withdraws from the study, the reason must be noted on the eCRF. Case report forms are to be completed on an ongoing basis.
Designated investigator staff will enter the data required by the protocol into the eCRF. The Investigator must certify that the data entered into the eCRFs are complete and accurate.
All data obtained using paper collection methods during the clinical study will be recorded in eCRF software. All source documents from which eCRF software entries are derived should be placed in the subject's personal records.
The original eCRF software entries for each subject will be checked against source documents by the study monitor. Instances of missing or uninterpretable data will be discussed with the investigator for resolution.

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.
Subject names will not be supplied to the sponsor. Only the subject number will be recorded in the eCRF, and if the subject name appears on any other document, it must be obliterated before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. As part of the informed consent process, the subjects will be informed in writing that representatives of the sponsor, EC or regulatory authorities may inspect their records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws. If the results of the study are published, the subject's identity will remain confidential. The Informed Consent Form (ICF) will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation. The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure and use of their data must also be explained to the subject in the informed consent
All clinical study findings and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the sponsor.

The anonymity of participating subjects must be maintained. Subjects will be specified in outputs and other documents containing subject data by their subject number, not by name. Documents that identify the subject (e.g., signed ICF) will be maintained in confidence by the investigator.

15.3.2 Database management and quality control

Data Management is the responsibility of Convex Ltd. With permission of the Sponsor, Data Management may be delegated under an agreement of transfer of responsibilities to a qualified vendor of Convex Ltd.
The full details of procedures for data handling will be documented in the Data Management Plan (DMP).
AEs and medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary.
Unique subject numbers will identify the subject and the biological material obtained from the subject. Appropriate measures such as encryption or deletion will be enforced to protect the identity of human subjects in all presentations and publications as required by local/regional/national requirements.
Data from screening failures will not be entered into the database.
Laboratory data from the safety laboratory will be provided to Convex Ltd via access controlled, password protected web directory. The results will be printed and signed in paper and these will be considered source data. In cases where sensitive non-PK laboratory data is transferred via non-secure electronic networks, data will be encrypted during transfer.
All PK and PD data will be transferred to the responsible Data Management party, whereby the transfer process will be pre-defined in data transfer agreements.

eCRFs will be developed by Data Management party in collaboration with the clinical study team and statistician. The Data Management party will document the process workflow in the Data Management Plan (DMP). After data entry, monitor(s) will verify the eCRFs against the source documents. Queries may be issued to clarify the data entered. The PI will electronically sign the eCRFs after all data have been entered and all queries have been resolved. If corrections and/or resolution of queries are required after PI approvals, those eCRFs affected by changes will be re-signed by the PI. The database may be locked after the PI approvals are completed.
The Investigator will receive all laboratory data electronically or based on printed reports directly from the laboratory. An Investigator must review, evaluate, sign and date the laboratory print-outs upon receipt.
After database lock, study design documentation and locked eCRFs (PDF) will be created and can be provided to the Sponsor, if requested.
Data required for analyses and subject safety assessments will be entered from source documentation into eCRFs. Instructions for data entry will be provided in the eCRF Completion Guidelines, developed by Convex Ltd Operational team. All site staff involved with entering data into the eCRFs will be trained prior to gaining access to the study database. Queries may be generated by the eCRF system during data entry, and queries may be generated by CDM staff, monitors, PIs, and other data reviewers during the course of the study. Only specific site personnel will be authorized to make corrections to the eCRFs; CDM will train personnel prior to granting access in the eCRF system. Corrections will be made directly in the eCRF – by modifying existing data, adding new data, or deleting data, as appropriate. All data corrections will be logged in the electronic audit trail.
The Investigator or Investigator's authorized staff must ensure that all information derived from source documentation is consistent with the source information and accurately reflected in the eCRFs. By electronically signing the eCRFs, the Investigator confirms that the information is complete and correct.

15.4 Access to source data and documents

Source data are the original records of all variables collected for the clinical investigation. They include, but are not limited to:

- Signed informed consent
- Laboratory reports, test analysis reports, procedures results for all interventions performed in accordance with the protocol
- Individual subject clinical notes
- Details concerning inclusion and exclusion criteria
- The medical history prior to participation in the study
- The basic identifying information, such as demographics, that link the subject's source documents with the CRFs
- All safety events (AEs, SAEs, etc.)
- All treatment exposure (prior, concomitant and test treatment)
- All relevant observations of the subject's health status during their participation in the study
- A description of interventions done to the subjects in the study

The Investigator must allow the monitor access to all documents in the subject's study file to confirm their consistency with the eCRF entries. All information entered in the eCRF must be available as source data. All study data will be handled confidentially. No information about subject identity on these documents will be allowed to leave the study site.

It is the responsibility of the Investigator to maintain a comprehensive and centralized filing system of all relevant documentation.

- Investigators will be instructed to retain all study records required by the Sponsor and regulatory authorities in a secure and safe facility with limited access for one of the following time periods based on notification from the Sponsor:
  - For a period of at least 2 years from the last marketing approval worldwide or for at least 25 years, whichever is the greater
  - Or a period of at least two years after discontinuation of clinical development of the investigational product as confirmed by the Sponsor
  - For a longer period if required by local regulations

The Investigator will be instructed to consult with the Sponsor before disposal of any study records and to provide written notification to the Sponsor of any change in the location, disposition, or custody of the study files.

The sponsor or its representative may visit the study facilities at any time in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, observation and discussion of the conduct and progress of the study. The investigator will permit trial-related monitoring, audits, EC review and regulatory inspections, providing direct access to source data and study documents.

15.5 Quality control and quality assurance

This study will be conducted according to applicable Standard Operating Procedures (SOPs) by Convex Ltd.
Before the first healthy volunteer is entered into the study, a Sponsor representative will review and discuss the requirements of the Protocol and related documents with the investigational staff and also train them in any study specific procedures and system(s) utilized, if applicable.

The PI will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved. The PI will maintain a record of all individuals involved in the study (medical, nursing and other staff).
Quality Management of Convex will periodically visit the Investigational site to discuss the conduct of the trial and upon necessity can audit the records of the trial. These reviews are necessary to ensure that the study is conducted according to standards consistent with the study protocol and ICH GCP Guideline.
The Investigator agrees to discuss and correct, if necessary, any problems or deficiencies that are found during the course of these reviews.

The Sponsor has the right to perform an external QA audit according to their internal procedures.

15.5.1 Monitoring

The study will be monitored by Sponsor's representative who will perform an initiation visit before the first subject is screened, have regular contacts with the study site, including routine visits conducted to:

- Provide information and support to the Investigator(s).
- Assure compliance with the study protocol.
- Verify that the research facilities, including laboratories and equipment, are adequate to safely and properly conduct the study.
- Verify that the investigational product is stored properly and under the proper conditions, is in sufficient supply, and that receipt, use, and return of investigational product are controlled and documented adequately.
- Verify that written informed consent was obtained before any protocol-specific screening procedures are performed solely for the purpose of determining eligibility for the clinical study and/or prior to the provision of study medication.
- Review the subject CRFs and source documents to ensure that reported study data are accurate, complete, and verifiable from source documents.
- Ensure that adequate records of clinical trial supplies are maintained.
- Verify that the Investigator and study site personnel are adequately qualified throughout the study.
- Verify that the safety information and amendments are submitted to the relevant authorities.

All checks that the data given in the eCRF complies with the source document will be carried out in accordance with the study specific monitoring manual.

The monitor will be available between visits if the Investigator(s) or other staff at the centre needs information and advice about the study conduct.

After the data base hard lock and approval from the Sponsor a close-out visit will be performed in order to close the study at the site.

15.6 Protocol amendments

Any change to a protocol has to be considered as an amendment. All protocol amendments must be approved by the Sponsor before they are submitted to EC and CA. All substantial amendments will be implemented after the receipt of a written approval from the EC and CA. All non-substantial amendments will be implemented after they have been notified to EC and CA.

15.6.1 Substantial amendment

Significant changes that affect subject safety and/or the scientific value of a trial require a substantial amendment. Examples of significant changes are given in EU guidelines on the request to the competent authorities for authorisation of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1, 2010/C 82/01). The need for submitting a substantial amendment is the responsibility of the sponsor. Substantial amendments are to be approved by the appropriate EC and the CA will need to provide a 'no grounds for non-acceptance' notification prior to the implementation of the substantial amendment.

15.6.2 Non-substantial amendment

Non substantial amendments do not affect subject safety or the scientific integrity of the trial. Non-substantial amendments will be approved (signed) by the investigator(s) and will be recorded and filed by the investigator/sponsor. Non-substantial amendments will be submitted to the EC for information only. The CA will only be notified by changes in Eudract form and ABR form (if applicable). The implementation of a non-substantial amendment can be done immediately.

The EU guideline CT-1 2010/C 82/01 stipulates the importance of preventing over-reporting. Therefore, the following changes are by definition non-substantial in this study:

- change in amount and timing of the samples (maximum of 2 samples without a > 50 mL increase in the amount of blood taken and not exceed 500 mL of blood in total)
- changes in assay-type and / or institution where an assay will be performed, provided that validated assays will be used;
- editorial changes to documents in the submission dossier including the subject information sheets and the protocol. An editorial change is defined as a modification in the documents of typographical errors and other modifications that in no way alter the meaning or content of the document;
- determination of additional parameters in already collected materials, which are in agreement with the study objectives and do not provide prognostic or genetic information;
- other statistical analyses than described in the protocol;
- a change in clinical staff, including the principal investigator, when this concerns regular staff members of the CRO who comply with internal regulations for training and authorisation.

15.6.3 Urgent amendment

An urgent amendment might become necessary to preserve the safety of the subjects included in the study. The requirements for approval should in no way prevent any immediate action being taken by the investigators or the sponsor in the best interests of the subjects. Therefore,

if deemed necessary, an investigator can implement an immediate change to the protocol for safety reasons. This means that, exceptionally, the implementation of urgent amendments will occur before submission to and approval by the EC(s) and CA.

15.7 Clinical study report

After completion of the study, the complete final study report will be prepared in a form based on ICH E3 Guideline: Structure and Content of Clinical Study Reports.
All individual data that allow re-calculation of pharmacokinetic parameters (concentrations, actual sampling) and pharmacokinetic parameters results will be available in electronic format and will be provided together with the report.

15.8 End of study report

The CRO on behalf of the sponsor will notify the EC and the CA of the end of the study within a period of 90 days. The end of the study is defined as the last subject's last visit.
In case the study is ended prematurely, the CRO will notify the EC and the CA within 15 days, including the reasons for the premature termination.
The CRO on behalf of the sponsor will notify the EC and CA immediately of a temporary halt of the study, including the reason of such an action.
The Principal Investigator will inform all study subjects in case of temporary halt and/or premature termination of the study.
Within one year after the end of the study, the responsible CRO will submit a synopsis of the clinical study report (CSR) with the results of the study, including any publications/abstracts of the study, to the EC and the CA. The principal investigator, CRO's representative and the sponsor's representative will be the signatories for the study report.

15.9 Public disclosure and publication policy

The principal investigator/Convex Ltd. may not publish the data collected during this study in any form, except with the written permission of the Sponsor. In case of publication the principal investigator will have the opportunity to review the analysis of the data and to discuss with the sponsor the interpretation of the study results prior to publication. Any study-related article or abstract containing partial or complete study results, written independently by investigators should be submitted to the sponsor for review at least 60 days prior to submission for publication or presentation. The list of authors of any formal publication or presentation of study results may include, as appropriate, representatives of the sponsor and will be determined by mutual agreement. In case of publication, confidentiality of the study subjects will be respected.

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17 ANNEXES

17.1 Annex No 1

SERIOUS ADVERSE EVENT REPORT
Send within 24 hours from awareness per email to:

[redacted]

SERIOUS ADVERSE EVENT REPORT

Sponsor:	
Protocol Number:	
Site Number:	
Country:	

Investigational Product:	
Principal Investigator:	
Subject Initials:	
Subject Number:	

1. Reporting Information:	**Date the Investigator/investigational team became aware of this SAE:**	
☐ **Initial**	**Date the initial report was received from the site:**	
	Date the Sponsor was notified:	

☐ **Follow-Up No_____**	**Date follow-up information was received from the site:**	
	Date the Sponsor was notified:	

2. Subject Information:			
Age at onset:	years	**Height:**	cm
		Weight:	kg
Sex:	☐ **Male**	☐ **Female**	

3. Serious adverse event information

Adverse event term* (provide a clinical diagnosis where possible):	_____ *State diagnosis when possible. In case of no confirmed diagnosis, document only leading sign, syndrome or preliminary diagnosis that presumably led to one of the seriousness criteria listed in the section 5. Please report 1 event term per line.
Onset date of SAE:**	_____ dd – mmm – yyyy **Document date when the symptom of SAE occurred first (for seriousness criteria see section 5.). In case of hospitalization document date of admission as "Onset Date"
Narrative* (please, use an additional sheet if necessary):** ***Report clinical signs and symptoms as well as any other supporting information that led to the diagnosis. Describe course of the event and any treatment of the event including details of medications and procedures. Please add rationale of your event causality assessment	

4. Severity of adverse event:		
☐ Mild	☐ Moderate	☐ Severe

5. Seriousness criteria (check the most appropriate criterion):

☐ Death	☐ Life-threatening	☐ Persistent or significant disability/ incapacity	☐ Congenital anomaly/birth defect	☐ Medically important event or reaction
☐ Hospitalization (initial ☐ or prolonged ☐)	Date of hospitalization: _____ dd – mmm – yyyy		Date of discharge: _____ dd- mmm – yyyy	

6. Event causality (relationship to the Investigational Product):				
☐ Certain	☐ Probable	☐ Possible	☐ Unlikely	☐ Not related

7. Study medication:	**Please, check the study period at the time of the event's onset**
☐ Screening ☐ Maintenance ☐ Titration ☐ Conversion or Tapering ☐ Additional Tapering ☐ Follow-up ☐ Other:_____	
Administration:	
☐ single ascending dose (SAD) part	☐ multiple-ascending dose (MAD) part
Dose level :	_____ _____ Dose Units
(First) Study Medication Intake**:** ****applicable for the SAD part and for the first dose of the MAD part	**Last Study Medication Intake*****:** (before the onset of the SAE) *****applicable for **the MAD part** of the study only
_____ dd – mmm – yyyy / _____ Hour/Min	_____ dd – mmm – yyyy / _____ Hour/Min
Did event alleviate after stopping the administration of the Study Medication?	☐ N/A ☐ No ☐ Yes

Did event reappear after reintroduction of the Study Medication?	☐ N/A ☐ No ☐ Yes

8. Action taken due to this event regarding the study medication:		
☐ None	☐ Discontinuation of the study drug Date: _____ dd – mmm – yyyy	☐ Use of intervention to treat the AE (provide details, recording concomitant therapy, if needed)
☐ Study drug dosing interrupted	Date: _____ dd – mmm – yyyy	Restart date:_____ dd – mmm – yyyy

9. Relevant medical history/co-existing diseases (please, use an additional sheet if necessary, or attach a copy/print-out of the Medical History CRF/eCRF)

☐ Please, check if an additional sheet is attached

10. Relevant tests/laboratory data (please, use an additional sheet if necessary, or attach a copy of the lab reports) Please provide the list of any abnormal laboratory or diagnostic tests relevant to this adverse event. Do not include information not pertinent to this event.

☐ Please, check if an additional sheet is attached

11. Concomitant medication/therapy at the time of the event (Alternatively, please attach a copy/print-out of the concomitant medication CRF/eCRF)

Drug name	**Start date (dd-mmm-yyyy)**	**Stop date (dd-mmm-yyyy)**	**Dose**	**Units**	**Route**	**Indication**

☐ Please, check if an additional sheet is attached

12. Final outcome of the serious adverse event:

☐ Recovered/resolved →	Date recovered/resolved: _____ dd – mmm – yyyy	
☐ Recovered/resolved with a sequelae →		
☐ Fatal →	Date of death: _____ → dd – mmm – yyyy	Specify the cause of death: _____ Autopsy report obtained ☐ Yes ☐ No
☐ Recovering/Resolving	☐ Not recovered/Not resolved	☐ Unknown

Signatures:	
Name of the reporter:	

Phone number: **Fax number:** **Email:**		
Reporter's signature:		**Date: _____** **dd – mmm – yyyy**

Investigator's name:		
Phone number: **Fax number:** **Email:**		
Investigator's signature:		**Date: _____** **dd – mmm – yyyy**

17.2 Annex No 2

PREGNANCY REPORT AND OUTCOME FORM FOR MALE SUBJECT'S PARTNER

Sponsor:		**Investigational Product:**	
Protocol Number:		**Principal Investigator:**	
Site Number:		**Subject Initials:**	
Country:		**Subject Number:**	

MATERNAL DATA

Patient date of birth (if possible in respect of Data Protection Law in your Country):
_____

Relevant medical history (risk factors, smoking, alcohol, etc):
_____

_____

Previous Pregnancies: _____ **Previous Normal deliveries: _____**
Previous Spontaneous miscarriages: _____

Relevant family history:

_____

_____

Last menstrual date: _____

Estimated date of delivery: _____

- Was the patient using hormonal contraceptive/IUD at time of conception? ☐ yes ☐ no

- Is there evidence of a defect from a prenatal test? ☐ yes ☐ no

If yes, indicate which test(s) showed evidence of birth defect:

☐ Ultrasound Amniocentesis

☐ Other, specify: _____

MEDICATION(S) TAKEN DURING PREGNANCY

Product name	Daily dose	Treatment duration (Start date/Stop date)	Indication

MEDICATION(S) TAKEN AT THE TIME OF CONCEPTION

Product name	Daily dose	Treatment duration (Start date/Stop date)	Indication

Signatures:

Name of the reporter:		
Phone number: **Fax number:** **Email:**		
Reporter's signature:		**Date: _____** **dd – mmm – yyyy**

Investigator's name:		
Phone number: **Fax number:** **Email:**		
Investigator's signature:		**Date: _____** **dd – mmm – yyyy**

PREGNANCY OUTCOME FORM

Sponsor:	
Protocol Number:	
Site Number:	
Country:	

Investigational Product:	
Principal Investigator:	
Subject Initials:	
Subject Number:	

PHOETUS/CHILD DATA

☐ **Elective termination on: _____**

☐ **Spontaneous miscarriage on: _____**

☐ **Premature birth - Date of birth: _____**

☐ **Full term - Date of birth: _____**

Birth weight: _____

Gender: ☐male ☐female

If premature, gestational age: weeks: _____ days: _____

Comments: _____

METHOD OF DELIVERY:

☐ **Normal vaginal** ☐ **Forceps** ☐ **Caesarean section**
☐ **Other, specify: _____**

OUTCOME

☐ **Healthy baby** ☐ **Sick baby (i.e., birth trauma, infection)** ☐ **Congenital anomaly/Birth defect** ☐ **Stillbirth**

Was a birth defect noted?..... ☐yes ☐no

IF SERIOUS ADVERSE EVENT DEFINITION IS MET, PLEASE REPORT THE EVENT AS SAE.

If any birth defects were noted, please describe:

_____

_____

To what do you attribute the defect(s):

_____

_____

Describe any immediately postnatal problems that occurred (i.e, jaundice, respiratory distress):

_____

_____

Additional comments:

_____ _____

Signatures:		
Name of the reporter:		
Phone number: **Fax number:** **Email:**		
Reporter's signature:		**Date: _____** **dd – mmm – yyyy**

Investigator's name:		
Phone number: **Fax number:** **Email:**		
Investigator's signature:		**Date: _____** **dd – mmm – yyyy**