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TITLE PAGE

CLINICAL TRIAL PROTOCOL

Open-Label Pilot Trial to Evaluate the Effects of Ilofotase Alfa on Biomarkers in Adult Patients with Hypophosphatasia

Sponsor code: AP-recAP-HPP-01-01

This trial will be performed in compliance with the principles of Good Clinical Practice (GCP).

CLINICAL TRIAL PROTOCOL

Open-Label Pilot Trial to Evaluate the Effects of Ilofotase Alfa on Biomarkers in Adult Patients with Hypophosphatasia

Protocol number	AP-recAP-HPP-01-01
CT number	2023-503186-35-00
Short title	Pilot trial of single dose ilofotase alfa in hypophosphatasia
Version	Version 1.1
Date of protocol	30-March-2023
Investigational product	Ilofotase alfa
Principal Investigator	Lothar Seefried, MD
Sponsor	AM-Pharma B.V. Stadsplateau 6 WTC Utrecht 3521 AZ Utrecht The Netherlands Tel: +31 30 228 9222

AUTHORIZATION OF CLINICAL TRIAL PROTOCOL

The Sponsor and the Principal Investigator agree to conduct the trial as outlined in this Clinical Trial Protocol. Any modification of the Clinical Trial Protocol must be agreed upon by the Sponsor and the Investigator and must be documented in writing.

Name/Position: | Date: (dd-mmm-yyyy) | Signature:

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30. MRZ. 2023

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PROTOCOL SYNOPSIS

Open-Label Pilot Trial to Evaluate the Effects of Ilofotase Alfa on Biomarkers in Adult Patients with Hypophosphatasia

Title (short)
Pilot trial of single dose ilofotase alfa in hypophosphatasia

Trial code
Sponsor code: AP-recAP-HPP-01-01

Sponsor
AM-Pharma BV
Stadsplateau 6 WTC Utrecht
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Trial center
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Objectives
Primary objective:
- The change in Inorganic Pyrophosphate (PPi), and Pyridoxal 5'-phosphate (PLP) levels after low, and high dose ilofotase alfa in adult hypophosphatasia (HPP) patients.

Exploratory objectives:
- To determine the safety and pharmacokinetic (PK) profile of a single iv dose of ilofotase alfa in adult HPP patients.
- To determine the pharmacodynamic (PD) profile of a single iv dose of ilofotase alfa in adult HPP patients.

Design and Treatments
This is a single-center, open-label, randomized, parallel group clinical trial in adult patients with HPP. Two different dose levels (0.8 mg/kg and 3.2 mg/kg) will be assessed. Up to twelve patients will be randomized, 6 patients to each trial arm. Patients will receive a single dose of 0.8 mg/kg or a single dose of 3.2 mg/kg. Ilofotase alfa will be administered as a 1-hour iv infusion on day 1.

Trial period
The trial period will include a run-in period of 2 days (day -2 to day -1) before administration of ilofotase alfa (day 1), followed by 9 days of observation after the ilofotase alfa administration (day 2 to day 10), with an additional follow-up assessment 14 days after ilofotase alfa administration (day 15).

Patients
Up to twelve (12) patients ≥ 18 years and ≤ 85 years of age with HPP.

Inclusion criteria
At screening, the following inclusion criteria must be met:
1. 18-85 years old, inclusive.
2. Genetically confirmed variant in the tissue-nonspecific isozyme alkaline phosphatase (ALPL)-Gene.
3. Clinical symptoms of HPP.
4. Medical history with 1) at least two independent measures of Alkaline Phosphatase (ALP) below lower level of normal (LLN) and 2) at least one measurement of either PPi or PLP above upper level of normal (ULN).
5. Provision of signed and dated informed consent form (ICF) in accordance with local regulations at screening.
6. Patients must agree not to get pregnant/not to get their partner pregnant, during the trial:

- Women of Child Bearing Potential must agree to use adequate and effective contraception means (criteria CTFG, 2020) for the period between trial drug administration and 14 days thereafter.
- Post-menopausal females not requiring hormonal contraception are defined as: no menses for 12 months without an alternative cause, and follicle stimulating hormone level must be used to confirm post-menopausal state in women not using hormonal contraception or hormonal replacement therapy (CTFG, 2020).
- Male subjects must agree to wear a condom during sexual intercourse and in case their partner is of child bearing potential they must agree to use adequate and effective contraception means (criteria CTFG, 2020) for the period between trial drug administration and 14 days thereafter.

Exclusion criteria

A patient who meets any of the following exclusion criteria at screening and/or baseline is excluded from participation in this trial:

1. Is unable or unwilling to participate in all scheduled visits and perform all protocol-mandated assessments.
2. Has a known or suspected hypersensitivity to ilofotase alfa or any components of the formulation used.
3. Body weight < 40 kilogram and > 120 kilogram.
4. Patient has a history of clinically significant abnormalities or of any illness that, in the opinion of the trial investigator, might confound the results of the trial or pose an additional risk to the patient by their participation in the trial.
5. NSAID use in the past 2 weeks.
6. Use of corticosteroids in the past 4 weeks.
7. Use of compounds intended to interfere with bone metabolism (e.g. Denosumab, Teriparatide, Romosozumab, Raloxifene) in the past 3 months.
8. Use of bisphosphonates in the past 2 years.
9. Participation in a drug trial within 60 days, or five times the half-life of the drug, whichever is longer, prior to administration of ilofotase alfa.
10. Use of asfotase alfa in the previous 3 months. Patients will not be withheld from approved asfotase alfa if medically indicated.
11. A patient who is currently pregnant or lactating.
12. Use of supplements including Vitamin B6.

Trial Drug, Dosage, and Route of Administration

Trial drug (ilofotase alfa) is provided in glass vials as a concentrate for infusion (aqueous buffer at a pH of 7.0). Prior to administration, the trial drug will be diluted with sterile sodium chloride 0.9% for injection (isotonic saline), United States Pharmacopeia (USP) / European Pharmacopoeia (EP) or equivalent, to a final volume of 50 mL and administered as an iv infusion using a dosing syringe. Trial drug will be administered as a 1-hour continuous iv infusion via a peripheral access point.

Prohibited Medication during the trial period

1. NSAIDs
2. Corticosteroids
3. Compounds intended to interfere with bone metabolism (e.g. Denosumab, Teriparatide, Romosozumab, Raloxifene)
4. Bisphosphonates
5. Supplements including Vitamin B6
6. Asfotase alfa

If there is a clinical indication to start any of these above medication, the subject should be withdrawn from the trial. Subjects who initiate any of the medication mentioned above during the trial period will be considered dropouts.

Criteria for evaluation

Primary endpoints

PPi and PLP serum concentration levels – recorded on day 1 (post dose) trough day 10 (inclusive).
A composite endpoint of the lowest serum concentration level of each of PPi and PLP will be created.

Exploratory endpoints

Pharmacokinetics:	ilofotase alfa serum concentrations measured between day 1 and day 10 (inclusive).
Safety:	Adverse events (AE) measured between day 1 and day 15 (inclusive).

	Vital signs (including supine systolic and diastolic blood pressure, pulse, body temperature, respiratory rate) measured at screening, on day 1, and on day 10. Clinical laboratory (including clinical chemistry, hematology, and urinalysis) tests, physical examination measured on day 1 and day 10 and listed in footnotes 6 and 7 of Table 1. Presence of anti-ilofotase antibodies detected on day 10.
Pharmacodynamics	Urine phosphoethanolamine (PEA) - measured between day 1 and day 10 (inclusive). Immediately consecutive sampling (second morning urine) of urine and blood for creatinine, phosphorus, and calcium concentrations to assess phosphate reabsorption (ratio of tubular maximum reabsorption of phosphate (TmP) to glomerular filtration rate (GFR)(TmP/GFR) and tubular reabsorption of phosphate (TRP)) kidney function and Ca/creatinine ratio. Blood samples for: Purines, 25 OH Vitamin D, alkaline phosphatase (ALP) activity and Isoenzymes, c-Terminal Fibroblast Growth Factor-23 (iFGF-23), parathyroid hormone (PTH), C-terminal telopeptide (CTX), procollagen type I N-propeptide (PINP), Osteocalcin, Vitamin B6 VitamerPL, phosphoethanolamine (PEA) in Urine (absolute and relative to sCreat) D measured between day 1 and day 10 (inclusive).

Statistical methods
The final database lock (DBL) will take place after the final patient has reached day 15 and the final PPi and PLP levels have been reported.

Primary endpoint
This analysis will use the intention to treat (ITT) analysis set.
Biochemical markers of HPP (PPi, and PLP) will be recorded for each patient at baseline (days -2, -1 and 1 before randomization) and after treatment, starting on day 1 and ending on day 10. PPi, and PLP levels will be listed and summarized for each of day 1 to day 10, overall and by trial arm. The median (minimum, maximum), mean, standard deviation (SD) and standard error of the mean (SEM)) PPi, and PLP levels will be described overall and by trial arm. These data will also be displayed graphically.
For both PPi, and PLP the mean change and percentage change from baseline (with SD) will be tabulated per study day and by trial arm and shown graphically. The baseline value will be defined as the median value of the measurements prior to randomization.
The maximum change and percentage from baseline (with SD) will be tabulated and shown graphically overall and by trial arm.

Exploratory endpoints.
Endpoints as listed under exploratory objectives will be listed and summarized, overall and by trial arm, using descriptive statistics by scheduled time point. No statistical tests will be performed on exploratory endpoints.

Safety
Safety will be assessed by examining the incidence of adverse events, serious adverse events and will be categorized by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT) and will be summarized by trial arm and overall. Physical examination findings will be reported by trial arm and overall using the safety analysis set (SAS). For adverse events, seriousness, severity, relationship to trial drug and whether leading to trial drug discontinuation or application of any emergency remedy will be displayed.

Anti-ilofotase alfa antibodies
Any patient who is found to have antibodies against ilofotase alfa measured at day 10 and showing an increase from baseline will be considered positive for antibodies. Tables showing the number (and percentage) of patients who were positive at baseline (by trial arm and overall) and who were positive by day 10 (by trial arm and overall) will be shown using the ITT analysis set. Also, a table showing the number of patients who became positive by day 10 having been negative at baseline will be shown (by trial arm and overall).

Table 1. Schedule of Assessments

Visit	Pre-Treatment		Treatment	Follow up									
Trial day	day -2	day -1	day 1[10]	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10 /early with-drawal [1]	day 15
Informed consent	X												
Eligibility check[2]	X		X										
Medical history	X												
Demographics	X												
Physical examination	X		X									X	
Body weight + height [3]	X		X										
Pregnancy test [4]	X		X										X
Defining FSH level[5]	X												
Safety laboratory	X		X									X	
12-lead ECG	X												
Vital signs	X		X									X	
Randomization			X										
Trial drug administration			X										
PK			X[6]	X	X	X	X	X	X	X	X	X	
PPi, PLP[7]	X	X	X[8]	X	X	X	X	X	X	X	X	X	
Urine samples [9]	X	X	X	X	X	X	X	X	X	X	X	X	
Exploratory biomarkers[10]	X	X	X	X	X	X	X	X	X	X	X	X	
ADA samples			X									X	
Previous and concomitant medication	X		X	X	X	X	X	X	X	X	X	X	
AE monitoring[11]			X	X	X	X	X	X	X	X	X	X	X

[1] Women of child bearing potential having day 10 assessment done on their early withdrawal date will need to be tested for pregnancy
[2] Check against inclusion and exclusion parameters
[3] Body weight and length will be used to calculate the body mass index (BMI), height only at screening
[4] Only for women of childbearing potential
[5] Only for postmenopausal women
[6] PK sampling on day 1 will include baseline, end of infusion, 2, 4 and 8 hours post infusion
[7] Samples to be drawn in the morning while fasting, for PPi and PLP (both Vitamers)
[8] Samples at baseline, 2, 4 and 8 hours after start dosing will be in non-fasted patients
[9] Immediately consecutive sampling (second morning urine) of urine and blood for PEA, creatinine, phosphorus, and calcium concentrations. To assess phosphate reabsorption (TmP/GFR and TRP) kidney function and Ca/creatinine ratio
[10] 25 OH Vitamin D, ALP Isoenzyme, c-Terminal iFGF-23, PTH, CTX, PINP, Osteocalcin, PEA Urine (absolute und relative to sCreat), Purines and other biomarkers
[11] This includes SAE reporting
[10] Baseline is defined as measurements taken on day -2, day -1 and day 1. Measurements taken on day 1 must be taken at any time up to immediately before randomization to be considered as baseline measurements. Measurements taken at or after randomization are not considered baseline measurements. Note that there is no day 0.

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LIST OF ABBREVIATIONS

ADA	Anti-drug antibodies
AE	Adverse event
AKI	Acute kidney injury
ALP	Alkaline Phosphatase
ALPL	tissue-nonspecific isozyme alkaline phosphatase
ALT (SGPT)	Alanine aminotransferase (serum glutamic-pyruvic transaminase)
AST (SGOT)	Aspartate aminotransferase (serum glutamic-oxaloacetic transaminase)
AUC	Area under the concentration-time curve
BMI	Body Mass Index
CIOMS	Council for International Organizations of Medical Sciences
CL	Clearance
CT	Clinical Trial
CTFG	Clinical Trial Facilitation Group
CTX	C-terminal telopeptide
CRO	Clinical Research Organization
DBL	Database lock
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
EC	Ethical Committee
ECC	Endogenous Creatinine Clearance
ECG	Electrocardiogram
eCRF	electronic Case Report Form
ELISA	Enzyme Linked Immunosorbent Assay
EP	European Pharmacopoeia
EU	European Union
GCP	Good Clinical Practice
Gamma-GT	Gamma glutamyl transferase
GPI	Glycosylphosphatidylinositol
GMP	Good Manufacturing Process
FSH	Follicle Stimulating Hormone
HPP	Hypophosphatasia
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
iFGF-23	c-Terminal Fibroblast Growth Factor-23
IMP	Investigational Medicinal Product
ITT	Intent to Treat
IRB	Institutional Review Board
IV	Intravenous
LDH	Lactate dehydrogenase
LLN	Lower Level of Normal
MAD	Multiple Ascending Dose
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical dictionary for regulatory activities
MSD	Meso Scale Discovery
NSAID	Non-Steroidal Anti-inflammatory Drug
PEA	Phosphoethanolamine
PINP	procollagen type I N-propeptide
PD	Pharmacodynamic
PK	Pharmacokinetic(s)
PKS	PK analysis sample
PLP	Pyridoxal 5'-phosphate
PopPK	Population PK

PPi	Inorganic Pyrophosphate
PT	Preferred Term
PTH	Parathyroid Hormone
ULN	Upper Level of Normal
SA-AKI	Sepsis Associated Acute Kidney Injury
SAE	Serious adverse event
SAD	Single Ascending Dose
SAS	Population for Safety Analysis
SD	Standard Deviation
SEM	Standard Error of the Mean
SOC	System Organ Class
TmP/GFR	ratio of tubular maximum reabsorption of phosphate (TmP) to glomerular filtration rate (GFR)
TRP	tubular reabsorption of phosphate
USP	United States Pharmacopeia
VD	Volume of distribution

1. INTRODUCTION

Recombinant human alkaline phosphatase (ilofotase alfa) is a full-length human chimeric alkaline phosphatase (ALP) that could benefit patients with hypophosphatasia (HPP), which is characterized by low activity of tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP).

1.1 Background

1.1.1 Alkaline Phosphatase and Hypophosphatasia

Hypophosphatasia is a rare, inherited, metabolic disease characterized by low activity of the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) biochemically characterized by an excess of extracellular inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP) [2]. Treatment of HPP with a recombinant TNSALP (asfotase alfa) has demonstrated to be very effective in the infantile form [3–6] and is currently in clinical investigation for the treatment of adults with juvenile onset HPP [1,7].

1.1.2 Recombinant Human Alkaline Phosphatase (ilofotase alfa)

Ilofotase alfa is a full-length human chimeric ALP derived by recombinant technology and produced in Chinese hamster ovary cells. Ilofotase alfa is encoded by a human intestinal ALP sequence wherein the sequence encoding the crown domain has been substituted with the corresponding human placental ALP sequence. The glycosylphosphatidylinositol (GPI) anchor has been removed resulting in a form that can be secreted. Ilofotase alfa has a projected mass of approximately 105 kDa based on the amino acid sequence derived from the deoxyribonucleic acid (DNA) sequence and approximately 130 kDa as a fully glycosylated molecule.

1.1.3 Clinical Studies with Recombinant Human Alkaline Phosphatase (ilofotase alfa)

In a Phase 1 single ascending dose (SAD) and multiple ascending dose (MAD) clinical trial in 51 healthy volunteers, all reported adverse events (AEs) were classified as mild or moderate and most were evenly distributed over the active and the placebo groups. The trial did not raise any safety concerns and single intravenous (iv) infusion of ilofotase alfa in the range of 0.32 mg/kg to 3.2 mg/kg (200 to 2000 U/kg), as well as daily iv infusions of ilofotase alfa at doses of 0.8 and 1.6 mg/kg (500 and 1000 U/kg) for three consecutive days, were well tolerated by healthy male and female patients.

A Phase 2a/2b Proof-of-Concept and Dose-Finding trial (STOP-AKI) with ilofotase alfa in 301 patients with SA-AKI (Sepsis Associated Acute Kidney Injury) has been completed. In Part 1 of STOP-AKI, patients were randomized to receive 0.4, 0.8, or 1.6 mg/kg of ilofotase alfa or placebo once a day for three consecutive days to identify the optimal dose. At the interim analysis, 1.6 mg/kg ilofotase alfa was selected by the Data Monitoring Committee (DMC) as the optimal ilofotase alfa dose for Part 2. In Part 2, 1.6 mg/kg ilofotase alfa was compared to placebo. The primary endpoint, the mean daily endogenous creatinine clearance (ECC) on Days 1 – 7 (area under the curve [AUC] 1-7 ECC), was not significant. However, ilofotase alfa showed a significant relative reduction in 28-day mortality of more than 40%. A positive effect on mortality was also seen in the 0.8 mg/kg dose. Overall, ilofotase alfa showed a long-term effect on the kidney function with significant improvement of ECC on day 21 and day 28 compared to day 1 and over the 28-day period. Non-fatal serious adverse events (SAEs) were comparable in the ilofotase alfa and placebo groups, indicating that the drug is well tolerated and safe.

A randomized, double-blind, placebo-controlled, two-arm parallel group, multi-center Phase 3 pivotal trial to investigate the efficacy and safety of ilofotase alfa for the treatment of patients with SA-AKI has recently been prematurely terminated (NCT04411472), in this trial no safety concerns were raised by the DMC.

A more detailed description of ilofotase alfa and results from non-clinical studies are provided in the Investigator Brochure (IB).

1.2 Rationale

Ilofotase alfa is a human recombinant enzyme replacement therapy that is hypothesized to be able to supplement deficient TNSALP and has demonstrated efficacy in animal models of HPP [8]. Ilofotase alfa has demonstrated to be effective and without safety concerns in adults with sepsis and acute kidney injury [9,10]. This is a pilot trial for a potential Phase 2/3 trial aimed at identifying whether treatment with ilofotase alfa can normalize circulating levels of PPi, PLP and other biochemical markers of TNSALP deficiency along with the safety/tolerability of different doses of ilofotase alfa.

1.2.1 Risk – Benefit

No specific benefit to the participants in this trial can be expected, however the study results are intended to improve the understanding of ilofotase alfa and its potential as a possible additional future treatment option.
Ilofotase alfa has been assessed in healthy volunteers and in adults with sepsis and acute kidney injury, without serious safety or tolerability observations. In addition, the risks to subjects in this study will be minimized by an adequate selection of the eligibility criteria and diligent close medical monitoring, including both clinical symptoms as laboratory assessments and wider parameters of the subjects.
Overall, risks are considered justified by the benefit of a potential additional future treatment option in HPP.

2. OBJECTIVES

Primary objective:

- The change in Inorganic Pyrophosphate (PPi) and Pyridoxal 5'-phosphate (PLP) levels after low and high dose of ilofotase alfa in adult HPP patients.

Exploratory objective:

- To determine the safety and pharmacokinetic (PK) profile of a single intravenous dose of ilofotase alfa in adult HPP patients.
- To determine the pharmacodynamic (PD) profile of a single intravenous dose of ilofotase alfa in adult HPP patients.

3. INVESTIGATIONAL PLAN

3.1 Trial Design and Plan

3.1.1 Trial design

This is a single-center, open-label, randomized, parallel group clinical trial in adult patients with HPP. Two different dose levels (0.8 mg/kg and 3.2 mg/kg) will be assessed. Up to twelve (12) patients will be randomized to one of two trial arms, 6 in each trial arm.
Patients will receive a single dose of 0.8 mg/kg or a single dose of 3.2 mg/kg. Ilofotase alfa will be administered as a 1-hour intravenous infusion on day 1.

3.1.2 Screening and Informed consent

Patients will report for the eligibility screening according to the inclusion and exclusion criteria defined in Section 3.2 within 6 weeks prior to the first drug administration.
Patients will voluntarily sign the trial specific Informed Consent Form (ICF) prior to any trial specific screening procedures being performed. The written informed consent will be obtained for all patients, regardless of their eligibility for the trial; the signed ICFs will be retained and archived at the trial center and another copy will be provided to the patient.
Eligibility screening will consist of assessments as presented in the Schedule of assessments Table 1.

3.1.3 Treatment Period

The patients will arrive at the clinical research center two days (day -2) before the drug administration (day 1 is the day of drug administration). Patients will leave the research center at the end of the observation period on day 10 after completion of all assessments (see Schedule of Assessments Table 1). Assessments will be performed during the treatment period as presented in the Schedule of Assessments (see Table 1).

3.1.4 Follow-up

The follow-up will be performed at day 15. The specific procedures that will be performed are presented in the Schedule of Assessments (see Table 1).

3.1.5 Discussion Trial Design

A randomized, open-label design was chosen to assist the medical assessment of whether there is a change in PPi, and PLP between the two different doses. HPP patients may have a history of elevated

serum levels in either PPi or PLP, or both. The doses selected, single doses of 0.8 mg and 3.2 mg, were also assessed in the single ascending dose studies in healthy volunteers AP-recAP-AKI-01-01 and AP-recAP-AKI-01-02 previously conducted. This dosing regimen has been administered to both healthy volunteers and SA-AKI patients without relevant safety observations. Since both doses administered in the current trial have been administered previously in other clinical trials, the groups can be dosed in parallel.

3.2 Selection of Trial Population

Up to 12 patients should be enrolled.

3.2.1 Inclusion Criteria

At screening, the following inclusion criteria must be met:

1. 18-85 years old, inclusive, at screening.
2. Genetically confirmed variant in the ALPL-Gene.
3. Clinical symptoms of HPP.
4. Medical history with 1) at least two independent measures of Alkaline Phosphatase (ALP) below lower level of normal (LLN) and 2) at least one measurement of PPi or PLP above upper level of normal (ULN).
5. Provision of signed and dated informed consent form (ICF) in accordance with local regulations at baseline.
6. Patients must agree not to get pregnant/not to get their partner pregnant, during the trial
    - Women of Child Bearing Potential must agree to use adequate and effective contraception means (criteria CTFG, 2020[11]) for the period between trial drug administration and 14 days thereafter.
    - Post-menopausal females not requiring hormonal contraception are defined as: no menses for 12 months without an alternative cause, and follicle stimulating hormone level must be used to confirm post-menopausal state in women not using hormonal contraception or hormonal replacement therapy (CTFG, 2020).
    - Male subjects must agree to wear a condom during sexual intercourse and in case their partner is of child bearing potential they must agree to use adequate and effective contraception means (criteria CTFG, 2020) for the period between trial drug administration and 14 days thereafter.

3.2.2 Exclusion Criteria

A patient who meets any of the following exclusion criteria at screening and/or baseline is excluded from participation in this trial:

1. Is unable or unwilling to participate in all scheduled visits and perform all protocol-mandated assessments.
2. Has a known or suspected hypersensitivity to ilofotase alfa or any components of the formulation used.
3. Body weight < 40 kilogram and > 120 kilogram.
4. Patient has a history of clinically significant abnormalities or of any illness that, in the opinion of the trial investigator, might confound the results of the trial or pose an additional risk to the patient by their participation in the trial.
5. NSAID use in the past 2 weeks.
6. Use of corticosteroids in the past 4 weeks.
7. Compounds intended to interfere with bone metabolism (e.g. Denosumab, Teriparatide, Romosozumab, Raloxifene) in the past 3 months.
8. Use of bisphosphonates in the past 2 years.
9. Participation in a drug trial within 60 days, or five times the half-life of the drug, whichever is longer, prior to administration of ilofotase alfa.
10. Use of asfotase alfa in the previous 3 months. Patients will not be withheld from approved asfotase alfa if medically indicated.
11. A patient who is currently pregnant or lactating.
12. Use of Supplements including Vitamin B6.

3.2.3 Removal of Patients from Therapy or Assessment

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

The Principal Investigator has the right to terminate participation of a patient for any of the following reasons: difficulties in obtaining blood samples, violation of the protocol, severe or serious adverse events (SAEs), or for any other reason relating to the patient's safety or integrity of the trial data.

In the event a patient is withdrawn from the trial, the Sponsor will be informed immediately. If there is a medical reason for withdrawal, the patient will remain under the supervision of the Principal Investigator until satisfactory health has returned.

If the Principal Investigator withdraws a patient for a trial drug related reason (according to the judgement of the Principal Investigator) he/she is considered a dropout.

The site will use best effort to ensure that non-completers and dropouts who have received trial drug complete the follow up assessments.

On withdrawal after dosing and prior to the scheduled Day 10 visit, the assessments of the Day 10 visit should be done accordingly.

3.3 Treatments

3.3.1 Treatments Administered

1-hour iv infusions of 0.8 mg/kg, or 3.2mg/kg ilofotase alfa.

3.3.2 Identity of Investigational Product

Specifications for trial drug administered are summarized in Table 2.

Table 2. Trial Drug Administered

Trial Drug Name	**ilofotase alfa**
Dosage Formulation	IMP is a clear, colorless to slightly yellow concentrate solution for infusion.
Unit Dose Strength(s)/Dosage Level(s)	Ilofotase alfa has an activity of approximately 5,000 U/mL (units of ALP activity) at 8.0 mg/mL (protein concentration) in an aqueous buffer at a pH of 7.0 containing: • 20 mM citrate • 250 mM sorbitol • 2 mM $MgCl_2$ • 50 µM $ZnCl_2$ The solution will be diluted with sterile sodium chloride 0.9% for injection (isotonic saline), USP/EP or equivalent and administered as an iv infusion to the intended doses. The intended dose will be in a volume of 50 mL.
Route of Administration	Intravenous infusion
Packaging and Labeling	For preparation of the trial drug, vials with 4 mL extractable volume of 8.0 mg/mL ilofotase alfa will be provided and labeled according to EU requirements.
Supplier	Almac Clinical Services Ltd.

3.3.3 Trial Drug Preparation

For preparation of the trial drug, vials with 4 mL extractable volume of 8.0 mg/mL ilofotase alfa will be provided. Prior to administration, the trial drug will be diluted with sterile sodium chloride 0.9% for injection (isotonic saline), USP/EP or equivalent, and administered as an iv infusion using an infusion line with an in-line filter and dosing syringe. The intended dose is 0.8 mg (500 U) per kg of body weight ilofotase alfa or 3.2 mg (2,000 U) per kg of body weight ilofotase alfa The intended dose will be in a volume of 50 mL.

Reconstituted trial drug can be stored at 2 to 8°C for up to 24 hours or for a maximum of 8 hours at room temperature and protected from light.
Details will be described in the Pharmacy Manual.

3.3.4 Trial Drug Administration

Only patients enrolled and randomized in the trial may receive trial drug. Start and stop time and date of each trial drug administration will be collected in the electronic case report form (eCRF). Trial drug is administered as an iv infusion using an infusion line with an in-line filter and dosing syringe. Infusion will take place at a constant rate of 50 mL/hour for 60 minutes.

3.3.5 Trial Drug Handling and Storage

Receipt and handling of trial drug will be in accordance with procedures at the site. Storage will be in a lockable, monitored storage facility with access limited to authorized staff only. Trial drug vials are to be stored at 2°C to 8°C and protected from light and moisture until preparation for use.
Full details of trial drug packaging, storage, and shipment will be provided in the Pharmacy Manual. Trial drug will be packaged and labeled in accordance with Good Manufacturing Practice (GMP), Good Clinical Practice (GCP) and local requirements.

3.3.6 Drug accountability

Current ICH GCP guidelines require the investigator to ensure that trial drug deliveries from the Sponsor are received by a responsible person (e.g. a pharmacist or delegate), and

- that such deliveries are recorded,
- that trial drug is handled and stored safely and properly,
- that trial drug is only dispensed to trial patients in accordance with the protocol,
- that any unused trial drug is returned to the Sponsor or standard procedures for the alternative disposition of unused trial drug are followed.
- Drug inventory and accountability records for the trial drugs will be kept by the investigator/pharmacist. Trial drug accountability throughout the trial must be documented. The following guidelines are therefore pertinent:
    i. The investigator agrees not to supply trial drugs to any person except the patients in this trial.
    ii. The investigator/pharmacist will keep the trial drugs in a pharmacy or other locked and secure storage facility under controlled storage conditions, accessible only to those authorized by the investigator to dispense these test drugs.
    iii. A trial drug inventory will be maintained by the investigator/pharmacist. The inventory will include details of materials received and a clear record of when they were dispensed and to which patient.
    iv. At the conclusion or termination of this trial, the investigator/pharmacist agrees to conduct a final drug supply inventory and to record the results of this inventory on the Drug Accountability Record. It must be possible to reconcile delivery records with those of used and returned medication. Any discrepancies must be accounted for. Appropriate forms of deliveries and returns must be signed by the person responsible.
    v. Unused drug will be locally destroyed according to standard institutional procedures after drug accountability has been conducted by the Sponsor or representative at the end of the trial. If such is not possible, unused drug will be returned to the Sponsor (or its designee).

3.3.7 Drug Storage

Storage at 2°C to 8°C, protected from light and will be indicated on the label.
The clinical supplies storage area at the site must be monitored by the site staff for temperature consistency with the acceptable storage temperature range specified by the Sponsor. Documentation of temperature monitoring should be maintained.

3.3.8 Method of Assigning Patients to Treatment Groups

After obtaining oral and written informed consent, patients will be screened according to the inclusion and exclusion criteria. Patients who have met all eligibility criteria receive a screening number upon inclusion in the trial. Replacement patients, for those patients who fail screening but were not randomized, will receive the screening number of the patient to be replaced, increased by 1000. Patients

who complete screening will be randomized to one of two trial arms, just prior to dosing, and will be given a subject number which will ensure identification throughout the trial.

Patients who dropout or withdraw for any reason before completing all screening evaluations successfully, will be considered as "screening failures". Such patients will not receive a subject number, but will receive a screening number, only applicable data will be entered in the eCRFs. The Principal Investigator or authorized designee will keep a screening and enrollment log of all patients screened to document patient numbers and passing screening status of enrolled patients into the treatment and patients who fail screening and the reasons for their exclusion.

3.3.9 Meals during the Trial

There are no special requirements related to food and beverage intake during the trial. Patients should abstain from alcohol from day -2 to day 10 of the trial.

3.3.10 Blinding

The trial is open label. Neither patients, clinicians, trial staff nor pharmacists will be blind to the trial drug.

3.3.11 Previous / Concomitant Medication and Other Restrictions during the Trial

The use of acetaminophen (paracetamol) is allowed. Other medication to treat AEs may only be prescribed after consultation of the Sponsor unless there is an emergency that does not allow discussion. In the event medication is used, the name of the drug, the dose and dosage regimen will be recorded in the eCRF.
Strenuous activity (activity resulting in muscle pains or perspiration) is not allowed from day-2 to day 10 of the trial.

3.3.12 Treatment Compliance

To ensure treatment compliance, medication dosing will be supervised by the Principal Investigator or his/her deputy. The exact times of medication dosing will be recorded in the eCRF. The actual volume dosed will be checked.

3.3.13 Prohibited Medication During the Trial (including the screening period)

1. NSAIDs
2. Corticosteroids
3. Compounds intended to interfere with bone metabolism (e.g. Denosumab, Teriparatide, Romosozumab, Raloxifene).
4. Bisphosphonates
5. Supplements including Vitamin B6
6. Asfotase alfa

If there is a clinical indication to start any of these above medication, the subject should be withdrawn from the trial. Subjects who initiate any of the medication mention above during the trial period will be considered dropouts.

3.3.14 Female subjects

Women of Child Bearing Potential (i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile) must have adequate and effective contraception means. Methods that are considered as highly effective birth control methods are: 1) Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal or transdermal), 2) progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), 3) intrauterine device (IUD), 4) intrauterine hormone-releasing system (IUS), 5) bilateral tubal occlusion, 6) vasectomised partner, 7) sexual abstinence. Contraception methods 3, 4, 5 and 6 have lower user dependency and should preferably be used, in particular when contraception is introduced as a result of participation in the clinical trial. (CTFG, 2020[11])

For post-menopausal female, with post-menopause defined as no menses for 12 months without an alternative medical cause, a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal

replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. (CTFG, 2020[11])

3.4 Sample collection

The Schedule of Assessments is presented in Table 1. All samples will be pseudonymized, collected and analyzed according to the instructions in the lab manual. Left over samples and samples for exploratory biomarkers will be bio-banked and stored at -80 °C in an appropriate facility. Unused samples will be retained for up to 10 years after completion of the trial and may be used for research purposes relating to HPP and/or the effects of ilofotase alfa on HPP disease parameters.

3.4.1 Primary endpoint samples

All samples to be obtained while fasting in the morning. There will be 3 samples obtained to determine baseline (day -2, day -1, and day 1 before randomization and dosing). There will be 12 samples obtained to determine changes in response to treatment (3 samples on day 1 *(2, 4, and 8 hours after start dosing*), and daily samples from day 2 until day 10.

3.4.1.1 PPi

All samples to be obtained while fasting in the morning and processed following the laboratory manual. Briefly, plasma samples will be centrifuged within 15 minutes of collection to yield platelet-depleted plasma, after which they will be stored at -80 °C until assessment according to the kit manufacturer instructions.

3.4.1.2 PLP

All samples to be obtained while fasting in the morning and processed following the laboratory manual. Briefly, all blood samples to be obtained while fasting in the morning, plasma samples will be centrifuged within 15 minutes of collection to yield platelet-depleted plasma, after which they will be stored at -80 °C until assessment according to the kit manufacturer instructions.

3.4.2 Exploratory assessments

Immediately consecutive sampling (second morning urine) of urine and blood for creatinine, phosphorus, and calcium concentrations to assess phosphate reabsorption (TmP/GFR and TRP) kidney function and Ca/creatinine ratio.

Blood samples for: 25 OH Vitamin D, ALP activity and Isoenzymes, iFGF-23, PTH, CTX, PINP, Osteocalcin, PL, and processed following the laboratory manual.
Blood sample for purine measurement processed following the laboratory manual.

For serum collection, blood samples will be allowed to clot for 30-60 minutes and then centrifuged for 15 min to separate the serum. For plasma sample collection, blood samples will be centrifuged within 15 minutes of collection to yield plasma. After collection of the serum/plasma in new tubes the samples will be stored at -80 °C until assessment.

Urine samples (for PEA, absolute and relative to Creatinine) will be collected as fresh as possible and transferred specimen from standard urine collection cup into the pre- labeled transfer tubes. After collection the urine samples will be stored at -80 °C until assessment.

3.5 Pharmacokinetic and Safety Variables

The Schedule of Assessments is presented in Table 1.

3.5.1 Pharmacokinetic Measurements

3.5.1.1 Ilofotase alfa concentration

Blood samples of 3 mL will be collected via an indwelling iv catheter or by a direct venipuncture at the time points indicated in Table 3 (see also Schedule of Assessments presented in Table 1).
The time clock (zero hours) starts at commencement of the iv dose of ilofotase alfa. The 60 minutes, 2-, 4- and 8-hours post dose blood samples may be collected within ±5 minutes of the scheduled times,

within ±60 minutes for all the other scheduled times. The exact times of blood sampling will be recorded in the eCRF.

Table 3. PK sample schedule for determination of ilofotase alfa

Pre-dose	**day 1**	Pre-dose (2 samples)
Post infusion	**day 1**	60 min (immediately post dose) 2h 4h 8 h
	day 2	24 h
	day 3	48 h
	day 4	72 h
	day 5	96 h
	day 6	120 h
	day 7	144h
	day 8	168 h
	day 9	192 h
	day 10	216 h

Note: The 60 minutes sample is to be taken from the arm NOT used for infusion.

PK samples will be analyzed by a central laboratory. Processing, storage, and shipment of the samples are described in detail in the Laboratory Manual.

3.5.2 Safety and Tolerability Measurements

Safety and tolerability assessments will consist of AEs, and results of vital signs, clinical laboratory tests, physical examinations, and anti-drug antibodies (ADA). Assessments will be performed in accordance with the schedule of assessments. The actual times of all assessments will be recorded in the eCRF.

3.5.2.1 Adverse Events

An AE is defined as any untoward medical occurrence in a patient administered a trial drug and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a trial drug, whether related to the trial drug.

At several time points before and after drug administration, patients will be asked non-leading questions to determine the occurrence of AEs. Patients will be asked in general terms about any AEs at regular intervals during the trial period. In addition, all AEs reported spontaneously during the trial will be recorded.

All answers will be interpreted by the Principal Investigator using the Medical Dictionary for Regulatory Activities (MedDRA), an internal validated medication dictionary for AEs and will be recorded in the AEs Record. Details on the rating of the intensity of the (S)AEs and relationship to the trial treatment are given in Appendix 7.1.

3.5.2.2 Vital Signs

During the trial, blood pressure and pulse will be recorded after 5 minutes of rest in the supine position and after 2 minutes of rest in the standing position. These assessments will be made using an automated device. Body temperature and respiratory rate will be measured subsequently or at the same time as blood pressure and pulse.

3.5.2.3 Electrocardiogram

At screening a standard 12 lead ECG will be recorded after 5 minutes of rest in the supine position using an ECG machine. The following ECG parameters will be recorded: heart rate, PR-interval, QRS-duration,

QT-interval, and QTc-interval (Fridericia's formula) together with the Principal Investigator's conclusion on the ECG profile. This ECG will serve as reference in case additional ECG are made on clinical indication.

3.5.2.4 Physical Examination

Physical examination will be performed according to the procedures of the trial center.

3.5.2.5 Anti-Drug Antibodies (ADA)

Blood samples of 4 mL will be taken at baseline (day 1) and at the end of the observation period (day 10) to determine the presence of antibodies against ilofotase alfa. The date and time of blood collection will be documented in the eCRF.

Immunological testing will be performed by a central laboratory by using an Enzyme Linked Immunosorbent Assay (ELISA) on a Meso Scale Discovery (MSD) platform. The assay is validated in human and septic serum samples. Processing, storage, and shipment of the samples are described in detail in the Laboratory Manual.

3.5.2.6 Clinical Safety Laboratory

Blood samples for clinical laboratory assessments will be collected at screening, day 1, and day 10, and analyzed locally according to the procedures of the trial center.

The following parameters will be measured:

Clinical chemistry (serum quantitatively):
total bilirubin, gamma glutamyl transferase (gamma-GT), aspartate aminotransferase (AST) (serum glutamic-oxaloacetic transaminase [SGOT]), alanine aminotransferase (ALT) (serum glutamic-pyruvic transaminase [SGPT]), lactate dehydrogenase (LDH), creatinine, blood urea, total protein (albumin and total), inorganic phosphate, sodium, potassium, calcium, glucose.

Hematology (blood quantitatively):
leucocytes, erythrocytes, hemoglobin, hematocrit, thrombocytes, partial automated differentiation: lymphocytes, monocytes, eosinophils, basophils, neutrophils, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

Urinalysis (urine qualitatively):
hemoglobin, urobilinogen, ketones, glucose, protein.

In the event of unexplained or unexpected clinical laboratory test values, the test(s) will be repeated as soon as possible and followed up until the results have returned to the normal range and/or an adequate explanation for the abnormality is found. The clinical laboratory will clearly mark all laboratory test values that are outside the normal range and the Principal Investigator will indicate which of these deviations are clinically significant. These clinically significant deviating laboratory results will then be recorded as AEs and the relationship to the treatment will be indicated (see also Appendix 7.1).

3.5.2.7 Pregnancy

Pregnancies will be recorded during the whole trial. Females who become pregnant during the trial must stop participation in the trial. In case of pregnancy the subject or female partners of male subjects will be followed-up until the pregnancy outcome is known. The Investigator must notify the Sponsor of any pregnancy.

The Investigator must actively follow-up, document and report on the outcome of all pregnancies, even if the subjects are withdrawn from the study.

3.5.3 Total of Blood Volume

Table 4 presents the number and volume of blood samples and the total volume of blood that will be collected throughout the trial per patient and trial part.

Table 4. Number and Volume of Blood Samples and Total Blood Volume Collected per Patient

Assessment	Maximum # samples	mL of blood per sample	Total volume of blood (mL)
Clinical laboratory	3	8	24
Clinical Chemistry			
Hematology			
ADA	2	4	8
Pharmacokinetics	15	3	45
ilofotase alfa			
Pharmacodynamics	15	14	210
Total volume of blood drawn			**287**

3.5.4 Timing of Assessments

The actual times of all assessments will be recorded in the eCRF.
For PK blood sampling, post dose blood samples may be collected within ±5 minutes of the scheduled time for samples the first 8 hours post dose, within ±60 minutes of the scheduled time for all other samples.
For safety assessments, pre-dose assessments on day 1 will also be performed between waking up and dosing. Sampling for clinical laboratory assessments at screening and post-dose at day 10 does not have a time-window.
In the event assessments are planned for the same scheme time, the order of the assessments should be arranged in such a way that PK blood sampling is done exactly on time, in practice generally vital signs will be done first, followed by blood sampling (exactly on time), followed by safety laboratory sampling.
Baseline is defined as measurements taken on day -2, day -1 and day 1. Measurements taken on day 1 must be taken at any time up to immediately before randomization to be considered as baseline measurements. Measurements taken at or after randomization are not considered baseline measurements.

3.6 Statistical Procedures

3.6.1 Trial Populations

Intention to Treat population (ITT)
All randomized patients will be included in the ITT population.

Population for the safety analysis (SAS)
All patients who have received trial medication.

Population for the PK analyses (PKS)
All patients who have received ilofotase alfa and for whom sufficient bioanalytical data are available to calculate reliable estimates of the PK parameters.

3.6.2 Statistical Evaluations

3.6.2.1 Primary endpoint

This analysis will use the intention to treat (ITT) analysis set.
Biochemical markers of HPP (PPi, and PLP) will be recorded for each patient at baseline (days -2, -1 and 1 before randomization) and after treatment, starting on day 1 and ending on day 10. PPi, and PLP levels will be listed and summarized for each of day 1 to day 10, overall and by trial arm. The median (minimum, maximum), mean, standard deviation (SD) and standard error of the mean (SEM)) PPi, and PLP levels will be described overall and by trial arm. These data will also be displayed graphically.
For both PPi, and PLP the mean change and percentage change from baseline (with SD) will be tabulated per study day and by trial arm and shown graphically. The baseline value will be defined as the median value of the measurements prior to randomization.

The maximum change from baseline will be tabulated (median, minimum, maximum, mean (including SD, SEM and 95% confidence interval)) and shown graphically (mean, SD and 95% confidence interval) overall and by trial arm.

3.6.2.2 Exploratory endpoints.

Endpoints as listed under exploratory objectives will be listed and summarized, overall and by trial arm, using descriptive statistics by scheduled time point. No statistical tests will be performed on exploratory endpoints.

3.6.2.3 Pharmacokinetic Evaluation

The PK serum concentration will be plotted versus time by treatment (individually, combined individual data and geometric mean). All individual data will be listed and described descriptively. The PK parameters and their statistical evaluation will be separate from the Clinical Trial Report of this trial. A separate analysis may include data of this trial in the existing population PK (PopPK) models to evaluate clearance (CL) and volume of distribution (Vd), which will be reported separately.

3.6.2.4 Evaluation of Safety and Tolerability Parameters

The Principal Investigator will evaluate the safety and tolerability of the treatment applied during the trial. This evaluation will consider the recorded AEs, vital signs, clinical laboratory, physical examination findings, and any other parameter that is relevant for safety assessment.

3.6.2.5 Adverse Events

All AEs reported will be coded and classified according to MedDRA. A listing will be given of all individual AEs. Summary tables of treatment emergent AEs (TEAEs) will be presented per treatment by system organ class and preferred term based on the MedDRA terminology list: one containing the number of TEAEs (frequency of occurrence, number and percentage of patients experiencing the event) by treatment and severity (intensity) and another containing the number of treatment related TEAEs (frequency of occurrence, number and percentage of patients experiencing the event) per treatment and severity. Additional total counts by treatment and relationship, and by treatment and severity will be given.

3.6.2.6 Vital signs

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

3.6.2.7 Physical Examination

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

3.6.2.8 Anti-Drug Antibodies

ADA data will be listed. Any patient who is found to have antibodies against ilofotase alfa measured at day 10 and showing an increase from baseline will be considered positive for antibodies. Tables showing the number (and percentage) of patients who were positive at baseline (by trial arm and overall) and who were positive by day 10 (by trial arm and overall) will be shown using the ITT analysis set. Also, a table showing the number of patients who became positive by day 10 having been negative at baseline will be shown (by trial arm and overall).

3.6.2.9 Clinical Laboratory Test Values

Summary statistics and plots will be generated for the change from baseline values, as deemed clinically appropriate. Summary statistics for the raw laboratory safety tests, may also be computed, as deemed clinically appropriate.

3.6.3 Determination of Sample Size

No formal sample size calculation is provided as the purpose of this trial is exploratory. Up to twelve patients will be randomized, 6 to each trial arm. If this randomization rate is not achieved in a reasonable timeframe, the Sponsor will review the required sample size.

3.7 Data Quality Assurance

The clinical research center will be monitored by the trial monitor to ensure correct performance of the trial procedures and assure that the trial will be conducted according to the relevant regulatory requirements.

By signing this protocol, the Sponsor agrees to be responsible for implementing and maintaining quality control and quality assurance systems with written procedures to ensure that trials are conducted, and data are generated, documented, and reported in compliance with the protocol, accepted standards of GCP and all applicable federal state and local laws, rules and regulations relating to the conduct of the clinical trial.

Regulatory authorities, the IRB/IEC and/or the Sponsor may request access to all source documents, eCRFs, and other trial documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must always provide support for these activities.

4. ETHICS

4.1 Regulatory and Ethical Considerations

The Investigator must promptly supply the Sponsor or its designee, the IRB/ IEC, competent authorities and, where applicable, the Trial Center, with written reports on any changes significantly affecting the conduct of the trial or increasing the risk to patients.
This trial will be conducted in accordance with the protocol and:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
- Applicable International Conference on Harmonization (ICH) GCP Guidelines.
- Regulation (EU) No 536/2014.
- Applicable laws and regulations.

The protocol, substantial protocol amendments, ICF, IB, and other relevant documents (e.g., any other written information regarding this trial to be provided to the patient or the patient's legal representative) must be submitted to the competent authorities and IRB/IEC and reviewed and approved by both institutions prior to being used in the trial, except for changes necessary to eliminate an immediate hazard to trial patients.

The Investigator will be responsible for the following:

- Providing written summaries of the progress and status of the trial to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
- Overall conduct of the trial at the site and adherence to requirements of ICH guideline for good clinical practice (E6(R2)) and applicable laws including but not limited to GDPR and applicable German privacy laws.
- Submitting reports of SAEs according to the timeline and method outlined in the protocol.
- Obtaining all required patient consents in accordance with applicable laws.

The Sponsor will cover this trial by means of an adequate insurance of the participating patients which will be in place prior to the start of the trial. As per local regulations, details about the insurance are described in the patient information sheet and informed consent form. A copy of the insurance certificate is filed in the Investigator Site File and the patient can request a copy.

4.2 Patient Information and Consent

All parties will ensure protection of patient personal data according to GDPR (and applicable German privacy laws) and will not include patient names on any forms, reports, publications, or in any other disclosures, except where required by laws.
The informed consent document used in this trial, and any changes made during the course of the trial, must be prospectively approved by the IRB/EC.
The investigator, or a person designated by the investigator, will obtain written informed consent from each patient before any trial specific activity is performed. The investigator and patient will each retain an original of the duly (by investigator and patient) signed ICF.
Prior to execution of the clinical trial, the Sponsor works together with the investigator to prepare the written ICF and other written information investigator. The written ICF and any other written information will be submitted to the competent authority according to the local requirements.

- The investigator is responsible for explaining the nature and purpose of the trial as well as other trial related matters to patients, using the written information. Also, the investigator is responsible for obtaining the patient's full understanding and written consent to participate in the trial of their own free will.
- The investigator or other responsible personnel who provided explanations (including collaborators who gave supportive information, if applicable) and the patient should sign and date the written information or write down his/her name and date the form.
- ICF must be obtained by the time that the first observations/examinations of the pre-investigational period are performed.
- The investigator or other responsible personnel should note the following when obtaining ICF from patients:
    - No patient may be subjected to undue influence, such as compulsory enrolment into a trial.

- The language and expressions used in the ICF should be as plain and understandable as possible. Patients should be given the opportunity to ask questions and receive satisfactory answers to the inquiry and should have adequate time to decide whether to participate in the trial. Written information should not contain any language or contents that causes the patients to waive or appears to waive any legal rights or that releases/mitigates or appears to release/mitigate the trial site, the investigator, collaborators, or the Sponsor from liability for negligence.

4.3 Data Protection

- Trial patients will be assigned a unique trial patients identification number. Any trial patients' records or datasets that are transferred to the Sponsor will contain this identifier only; trial patients names and any information which would make the trial patients identifiable will not be transferred. All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain trial patients' confidentiality.
- The trial patients must be informed that the patients' personal trial-related data will be used by the Sponsor in accordance with applicable data protection laws as stated in the ICF. The level of disclosure must also be explained to the trial patients as well as the patients' rights including the right to access or erase his/her personal data.
- The trial patients must be informed that the patients' medical records may be reviewed by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- All records will be kept in a secure storage area with limited access.

4.4 Patient Confidentiality

Individual patient medical information obtained because of this trial is considered confidential and disclosure to third parties is prohibited. Such medical information may be given only after approval of the patient to the patient's physician or to other appropriate medical personnel responsible for the patient's well-being.

The Sponsor, its board members, and its personnel shall not disclose any confidential information on patients obtained during the performance of their duties in the clinical trial without justifiable reasons.

All individuals and organizations involved in the trial must pay very careful attention to protect patients' privacy with appropriate measures, for example, by prohibiting the use of any private information that may identify a patient (e.g., name or address). These details shall be processed in accordance with GDPR and the applicable local and regional laws.

Even though any individuals involved in the trial, including the trial monitors and auditors, may get to know matters related to patients' privacy due to direct access to source documents, or from other sources, they may not disclose the content to third parties other than those designated by the Sponsor.

5. DOCUMENTATION

5.1 Archiving

Unless other Union law requires archiving for a longer period, the Sponsor and the Principal Investigator shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical trial. However, the medical files of subjects shall be archived in accordance with national law.

5.2 Recording of Data in Source Documents and eCRFs

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

A Data Management Plan will be written by the Sponsor or delegate which will be finalized prior to the first dosing.

6. CONFIDENTIALITY AND PUBLICATION POLICY

By signing this protocol, the Principal Investigator reaffirms to the Sponsor that the Investigator will maintain in confidence all information furnished to the Investigator or resulting from this trial. The Investigator will only divulge such information as may be necessary to the IRB/IEC and the members of the staff and the patients who are involved in this trial.

The Sponsor supports publication of the trial results, regardless of the outcome. It is understood that all data related to the trial remains the sole and exclusive property of the Sponsor. The Trial Center and/or Investigator may publish the results from the Trial; provided, however, that the Trial Center and/or Investigator submits the proposed publication to the Sponsor for review at least sixty (60) days prior to the date of the proposed publication. The Sponsor shall have the right to request modifications or removal from the proposed publication any Sponsor Information. The Trial Center and/or Investigator shall remove such Sponsor Information from the publication but only to the extent that doing so does not interfere with the complete and accurate presentation and interpretation of the Trial results. The Trial Center and/or Investigator shall consider such Sponsor's other comments in good faith but has no obligation to incorporate them. If such publication may affect the patentability of any invention to which the Sponsor has rights, the Sponsor shall have the right to request an additional delay to the proposed disclosure of no more than hundred-twenty (120) days so as to allow Sponsor to preserve its intellectual property.

7. APPENDICES

7.1 Adverse Events and Serious Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Adverse Event Definition
• An adverse event (AE) is any untoward medical occurrence (e.g., symptom, sign, diagnosis, or diagnostic test finding) in a patient enrolled into a clinical trial regardless of its causal relationship to trial drug. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated).

Events Meeting the Adverse Event Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., electrocardiogram [ECG], radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after trial drug administration even though it may have been present before the start of the trial. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either trial drug or a concomitant medication.

Events NOT Meeting the Adverse Event Definition
• Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the trial that do not worsen.

Treatment Emergent Adverse Event Definition
• A treatment emergent adverse event (TEAE) is defined as any event not present before exposure to trial drug or any event already present that worsens in either intensity or frequency after exposure to trial drug up to 14 days after last drug exposure.

Definition of Serious Adverse Event

An SAE is defined as any untoward medical occurrence that, at any dose:
Results in death
Is immediately life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

Requires inpatient hospitalization or prolongation of existing hospitalization

In general, hospitalization signifies that the patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE must be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

Results in persistent disability/incapacity

The term disability means a substantial disruption of a person's ability to conduct normal life functions.

This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

Is a congenital anomaly/birth defect

Other situations

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Medical or scientific judgment must be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Assessment of Severity

The severity of an AE refers to the extent to which an AE affects the patient's daily activities. The Investigator will assess severity for each AE and SAE reported during the trial and assign it to one of the following categories:

- Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe must not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

An event is defined as 'serious' when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the event must be noted for that day. Any change in severity of signs and symptoms over a number of days will be captured by recording a new AE, with the amended severity grade and the date (and time, if known) of the change. Changes in the severity of an AE must be documented to allow an assessment of the duration of the event at each level of intensity to be performed.

Assessment of Causality

- The Investigator is obligated to assess the relationship between trial drug and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to trial drug administration will be considered and investigated.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor or its designee. However, **it is very important that the Investigator always assesses causality for every event before the initial transmission of the SAE data to the Sponsor or its designee.**
- The Investigator may change his/her opinion of causality considering follow-up information and send an SAE follow-up report with the updated causality assessment.

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

- The relationship or association of the trial drug in causing or contributing to the AE will be characterized using the following classification and criteria:

Unrelated:	This relationship suggests that there is no association between the trial drug and the reported event.
Possible:	This relationship suggests that treatment with the trial drug caused or contributed to the AE; i.e., the event follows a reasonable temporal sequence from the time of drug administration or follows a known response pattern to the trial drug but could also have been produced by other factors.
Probable:	This relationship suggests that a reasonable temporal sequence of the event with trial drug administration exists and based upon the known pharmacological action of the trial drug, known or previously reported adverse reactions to the trial drug or class of drugs, or judgment based on the Investigator's clinical experience, the association of the event with the trial drug seems likely. The event disappears or decreases on cessation or reduction of the dose of trial drug.
Definite:	This relationship suggests that a definite causal relationship exists between trial drug administration and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event. The event reappears or worsens if the trial drug is re-administered.

Recording and Follow-up of Adverse Events and Serious Adverse Event

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in accordance with this trial's safety reporting documentation.
- It is **not** acceptable for the Investigator to send photocopies of the patient's medical records to the Sponsor or its designee in lieu of completion of the AE/SAE documentation.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor or its designee. In this case, all patient identifiers, except for the patient number, will be blinded on the copies of the medical records before submission to the Sponsor or its designee.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms or related procedure), as well as indication for the procedure, will be documented as the AE/SAE.

- All AEs must be reported in detail on the appropriate page of the eCRF.
- Follow-up of AEs will continue until resolution, stabilization, or death. In case of ongoing aEs at the moment of database closure, the data obtained at the moment of database closure will be used in the statistical analysis. The follow-up of the AE will be documented in the source documents and will be described in the final report only if considered relevant by the Principal Investigator.
- SAEs will be collected from Screening until the final trial visit. SAEs that are related to the investigational drug and continue beyond the normal collection period (i.e., are ongoing at the time a patient exits the trial) will be followed until resolution or until stabilized with sequelae. SAEs that begin after the patient's participation in the trial is complete, but that the Principal Investigator considers to be related to trial medication, may be reported at any time.
- The Principal Investigator or clinical site personnel should notify the Sponsor via email (SAEIntake@labcorp.com) or fax (0800 6648857) regardless of relationship to the investigational drug, within 24 hours of clinical site personnel becoming aware of the event. The Principal Investigator will provide the initial notification by forwarding a completed "SAE Notification Form" or the relevant, which must include the Principal Investigator's assessment of the relationship of the event to investigational drug.
- New or updated information will be recorded in the originally completed eCRF.
- The Investigator will submit any updated SAE data to the Sponsor or its designee within 24 hours of receipt of the information.
- The Sponsor or its designee will review each SAE report and the Sponsor, or its designee will evaluate the seriousness and the causal relationship of the event to trial drug. In addition, the Sponsor or its designee will evaluate the expectedness according to the IB. Based on the Investigator and Sponsor's assessment of the event, a decision will be made concerning the need for further action.
- The Sponsor or its designee will be responsible for all information processing and reporting according to local legal requirements.
- Contacts and instructions for SAE reporting can be found in the trial's safety reporting documentation.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

Any AE that is serious, associated with the use of the trial drug, and unexpected, i.e., not listed in current IB as expected for this trial (SUSAR), has additional reporting requirements, as described below.

- If the SUSAR is fatal or life-threatening, associated with trial drug, and unexpected, regulatory authorities and IRBs/IECs will be notified within 7 calendar days after the Sponsor, or its designee learns of the event. Additional follow-up (cause of death, autopsy report, and hospital report) information must be reported within an additional 8 days (15 days total).
- If the SUSAR is not fatal or life-threatening but is otherwise serious, associated with trial drug, and unexpected, regulatory authorities and IRBs/IECs will be notified within 15 calendar days after the Sponsor or its designee learns of the event.

The Sponsor or its designee will notify the Investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of patients. Follow-up information may be submitted if necessary.

The Sponsor will also provide annual safety updates to the regulatory authorities and IRBs/IECs responsible for the trial. These updates will include information on SUSARs and other relevant safety findings.

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