CLINICAL TRIAL PROTOCOL
Sponsor9s Reference Number: **ORPH-131-012**
Richmond Pharmacology Trial Number: **C23030**
IRAS Number: **1009060**

TITLE:	A Phase I, Single Centre, Randomised, Interventional, Open-Label, Cross-Over Study to Evaluate the Pharmacokinetics (PK) and the Safety and Tolerability of a Total Daily Dose of 900mg of TETA 4HCL, Comparing a New Once Daily TETA 4HCL Formulation (300mg) (3x300mg Trientine Base Tablets, OD) with the Current Marketed Cuprior® Formulation (150mg) (3x150mg Trientine Base Tablets, BD) in Adult Healthy Male and Female Participants
PHASE:	Phase 1
DRUG:	Trientine tetrahydrochloride (TETA 4HCL)
SPONSOR:	Orphalan SA 226 Boulevard Voltaire 75011 Paris, France
PRINCIPAL INVESTIGATOR:	Dr Thomas Ashdown MBBCh BSc Associate Medical Director
CO-PRINCIPAL INVESTIGATOR:	Dr Ulrike Lorch MD FRCA FFPM Chief Medical Director
TRIAL CENTRE:	Richmond Pharmacology Ltd. 1a Newcomen Street, London Bridge London SE1 1YR UK Telephone: +44 (0)20 7042 5800 Fax: +44 (0)20 7378 9135
Protocol Date:	Version 2.0, 09/Jan/2024

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PROTOCOL APPROVAL SIGNATURES

Version 2.0, Dated 09-Jan-2024

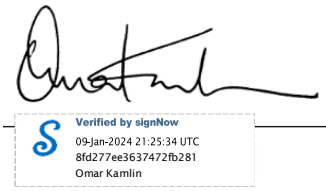
Sponsor9s Approval

This protocol has been approved by **Orphalan SA.**

Sponsor9s Signatory:

Name: **Dr Omar Kamlin MD FRACP FRCPCH DMedSci**
Senior Medical Director

Date: DD-MMM-YYYY

Signature: 

09-JAN-2024

INVESTIGATOR'S AGREEMENT

I have read this **Orphalan SA** Protocol No. **ORPH-131-012:**

A Phase I, Single Centre, Randomised, Interventional, Open-Label, Cross-Over Study to Evaluate the Pharmacokinetics (PK) and the Safety and Tolerability of a Total Daily Dose of 900mg of TETA 4HCL, Comparing a New Once Daily TETA 4HCL Formulation (300mg) (3x300mg Trientine Base Tablets, OD) with the Current Marketed Cuprior Formulation (150mg) (3x150mg Trientine Base Tablets, BD) in Adult Healthy Male and Female Participants

I have fully discussed the objectives of this trial and the contents of this protocol with the Orphalan SA representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical/regulatory review of the trial, without written authorisation from Orphalan SA. It is, however, permissible to provide information to a participant to obtain consent.

I agree to conduct this trial according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with ICH guidelines on GCP and with the applicable regulatory requirements.

I understand that Orphalan SA may decide to suspend or prematurely terminate the trial at any time for any reason as described in the Master Service Agreement (MSA) or Work Order with the clinical trial site; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial I will communicate my intention immediately in writing to Orphalan SA.

Principal Investigator:

Dr Thomas Ashdown MBBCh BSc

s;gnaturec **Date:** 10 - Jan - 2024

Co-Principal Investigator:

Dr Ulrike Lorch MD FRCA FFPM

Signature: **Date:** 10 ;)mu zoz<;-

TRIAL PERSONNEL

Sponsor9s Representative	Carla Bennett Principal Clinical Research Manager
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Pharmacovigilance	Richmond Pharmacology Ltd.
Medical Writing	Richmond Pharmacology Ltd.

LIST OF ABBREVIATIONS

For the purposes of this protocol, 8Investigator9 refers to the Principal Investigator or their delegate.

Abbreviation	**Explanation**
ABPI	Association of the British Pharmaceutical Industry
ADR	Adverse Drug Reaction
AE	Adverse Event
AR	Adverse Reaction
BHCG	Beta-hCG
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
CRF	Case Report Form
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CV	Coefficient of Variation
DHP	Data Handling Protocol
DSUR	Development Safety Update Report
ECG	Electrocardiogram
ET	Early Termination
eGFR	Estimated Glomerular Filtration Rate
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
HR	Heart Rate

IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
LFT	Liver Function Test
LLOQ	Lower Limit of Quantification
MSA	Master Service Agreement
MTS	Master Treatment Schedule
PI	Principal Investigator
PK	Pharmacokinetics
QC	Quality Control
QP	Qualified Person
RP	Research Physician
RPL	Richmond Pharmacology Ltd
RSI	Reference Safety Information
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SD	Standard Deviation
SmPC	Summary of Product Characteristics
SOA	Schedule of Assessments
SOC	System Organ Class
SOM	Study Operations Manual
SUSAR	Suspected Unexpected Serious Adverse Reaction
TETA	Trientine
TETA 2HCL	Trientine Dihydrochloride
TETA 4HCL	Trientine Tetrahydrochloride

ULN	Upper Limit of Normal
WD	Wilson9s Disease
WOCBP	Women Of Childbearing Potential

TRIAL SYNOPSIS

Protocol Reference: ORPH-131-012 **Study drugs:** TETA 4HCl

Title of the study: A Phase I, Single Centre, Randomised, Interventional, Open-Label, Cross-Over Study to Evaluate the Pharmacokinetics (PK) and the Safety and Tolerability of a Total Daily Dose of 900mg of TETA 4HCL, Comparing a New Once Daily TETA 4HCL Formulation (300mg) (3x300mg Trientine Base Tablets, OD) with the Current Marketed Cuprior® Formulation (150mg) (3x150mg Trientine Base Tablets, BD) in Adult Healthy Male and Female Participants

Principal Investigator: Dr Thomas Ashdown MBBCh BSc
Co 3 Investigator: Dr Ulrike Lorch MD FRCA FFPM

Study centre:

Richmond Pharmacology Ltd., 1A Newcomen Street, London Bridge, London SE1 1YR, UK

Study parts: N/A **Clinical phase:** Phase 1

Objectives	Endpoints	Statistical analyses
Primary		
To evaluate and compare the plasma PK parameters of TETA 4HCL and its two main metabolites (N1-acetyltriethylenetetramine (MAT) and N1, N10-diacetyltriethylenetetramine (DAT)) following administration of a total daily dose of 900mg TETA 4HCL, comparing the new once daily TETA 4HCL formulation (3x300mg trientine base tablets, OD) with the current marketed Cuprior® formulation, (3x150mg trientine base tablets, BD) in healthy adult male and female participants.	PK parameters derived by non-compartmental methods including maximum observed plasma concentration (Cmax), time to reach maximum plasma concentration (tmax), area under the plasma concentration-time curve from time zero to last detectable plasma concentration (AUC0-t), area under the plasma concentration-time curve from time zero to 24 hours plasma concentration (AUC0-24), area under the plasma concentration-time curve from time zero extrapolated to infinite (AUC0-¥), apparent total plasma clearance (CL/F), apparent volume of distribution during the terminal phase (Vz/F), terminal elimination rate constant (»z), and terminal elimination half-life (t1/2).	” Plasma concentrations will be listed and summarised by time point. Individual and arithmetical mean plasma concentration vs time curves for each analyte which includes all treatments will be produced on both linear/linear and log10/linear scales. ” The PK parameters will be listed for each participant and summarized for each treatment group using descriptive statistics (N - the number of participants, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum). ” For comparability of the two products the geometric mean, ratio of geometric means, confidence intervals (CI) including their logarithmic transformation together with the coefficient of variation for the within-participant variability will be summarised. In case a gender effect is noticeable, gender will be added as an additional term in the PK model, and all PK analyses will be presented per gender as well.

Secondary		
To compare the safety and tolerability of the two TETA 4HCL tablet formulations.	″ The incidence, severity, and relationship of Treatment-Emergent Adverse Events (TEAEs). ″ Proportion of participants with clinically significant changes in laboratory safety tests (haematology, biochemistry, coagulation, and urinalysis). ″ Proportion of participants with morphological and/or rhythm abnormalities on electrocardiogram (ECG). ″ Proportion of participants with clinically significant changes in vital signs (systolic blood pressure, diastolic blood pressure and pulse rate).	″ Adverse Events (AE), Serious Adverse Events (SAEs), vital signs, ECG parameters, and clinical laboratory data will be listed and summarised using descriptive statistics. ″ All AEs will be summarised and listed by using System Organ Class (SOC) and Preferred Term (PT) assigned to the event using Medical Dictionary for Regulatory Activities (MedDRA). Furthermore, these events will be summarised by the maximum intensity. The number of participants who experienced drug-related AEs will also be summarised. Any SAEs and/or AEs that led to withdrawal will be summarised and listed.

Study design

This is a single centre, phase I, randomized, controlled trial with a crossover design to evaluate the pharmacokinetics (PK), safety, and tolerability of a new once daily TETA 4HCL formulation (300mg) (3x300mg trientine base tablets, OD) compared to the current marketed Cuprior® formulation (150mg) (3x150mg trientine base tablets, BD) in adult healthy male and female participants.

Participants: 26 healthy participants will be enrolled to ensure 24 participants complete the study, with a balanced gender split.

Treatment: Participants will be randomized to receive either the new or the current formulation of the drug, and then switch to the other formulation after a period of time (see study flow chart below).

To remain in line with current EU SmPC and US PIL, being:

- EU daily dose range of 450-975 mg of trientine base
- US daily dose range of 150-1500mg trientine base

the following treatments will be administered according to the treatment allocation schedule below:

A: 900mg TETA 4HCl once a day / new formulation = 3 tablets of 300mg trientine base as a single dose

B: 900mg TETA 4HCl marketed Cuprior formulation = 6 tablets of 150mg trientine base in two equally divided doses

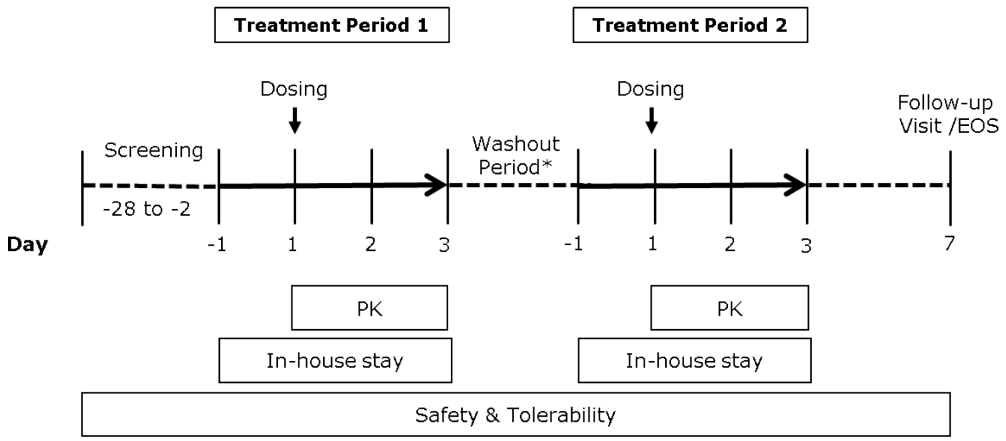
Patients will be randomised in a 1:1 ratio to either one of the following sequences:

Treatment Sequence	**Period 1**	**Period 2**
Sequence 1	Treatment A	Treatment B
Sequence 2	Treatment B	Treatment A

Assessments: Participants will be assessed for eligibility criteria and will be monitored closely throughout the study. Assessments will be performed during the study and at the end of the study follow-up visit.

Duration: The duration of the study will be up to approximately 7 weeks, from screening to follow-up:

- Screening will take place between days -28 and -2
- In-house period from D-1 to D3 with dosing on D1 of each treatment period
- Follow-up will take place on D7 of Treatment Period 2



* At least 5 days and a maximum of 10 days between treatment period study drug administrations

Objective: To evaluate the PK, safety, and tolerability of the new once daily TETA 4HCL formulation compared to the current marketed Cuprior® formulation.

Main criteria for inclusion:

- Age: 18 to 40 years
- Body weight: g 50 kg
- BMI: 18.0 to 25.0 kg/m2
- Health: Generally healthy, with no clinically significant illnesses or surgeries in the past 12 weeks
- Willingness to comply with trial procedures and restrictions

Participants must also be able to provide written informed consent before participating in the trial.

Main criteria for exclusion:

- Significant current or recurrent disease
- Acute significant disease or illness within 7 days before the start of the trial
- Clinically significant deviations in blood tests
- An estimated glomerular filtration rate (eGFR) of less than 60 ml/min/1.73m2
- Positive test for alcohol, drugs of abuse, hepatitis B surface antigen (HBsAg), hepatitis C antibody (HCV Ab), or human immunodeficiency virus antibody (HIV Ab)
- Pregnant or breastfeeding women
- History or regular use of tobacco or other nicotine-containing products within 6 months before the start of the trial
- Treatment with an investigational drug within 90 days or 5 half-lives (whichever is longer) or exposure to more than 3 investigational drugs within 12 months of first study drug administration
- Use of prescription medication (excluding female hormonal contraception or hormone replacement therapy)

within 30 days or 5 half-lives (whichever is longer) prior to first study drug administration, or use of over-the-counter (OTC) medication (including multivitamin, herbal, or homeopathic preparations; Paracetamol use f2g per day is permitted) during the 14 days or 5 half-lives of the drug (whichever is longer) before first study drug administration

- History of sensitivity/allergy to the study medications or components thereof (mannitol, colloidal anhydrous silica, glycerol dibehenate or magnesium-stearate)
- Donation or loss of 450 mL or more of blood or plasma within 16 weeks prior to first trial medication administration or intention to donate blood in the 16 weeks after completing the trial
- An inability to follow a standardised diet and meal schedule or inability to fast, as required during the trial
- Participants deemed to have difficult veins for cannulation/blood draws

These exclusion criteria are designed to protect the safety of participants and to ensure that the results of the trial are accurate and reliable.

1. INTRODUCTION

1.1 Background

Wilson9s Disease (WD) is an autosomal recessive life-threatening disorder caused by a mutation in the gene that codes for the ATP7B transport protein located on Chromosome 13[1]. The ATP7B protein mediates the binding of copper molecules to apoceruloplasmin in hepatocytes, forming ceruloplasmin, which can then safely transport the bound copper to its intended sites. In addition, the ATP7B protein also serves to transport excess copper from the hepatocytes into the bile for subsequent biliary excretion, therefore also permitting the safe elimination of excess copper. Such copper transport systems are essential, as although copper is fundamental for cellular function, free copper is toxic and causes cellular damage.

Pathogenic mutations in the ATP7B gene result in pathological copper accumulation, causing a wide spectrum of symptoms. WD presents symptomatically at any age, with the majority between 5 and 35 years[2]. Hepatic symptoms are the initial clinical manifestation in about 40-50% of WD patients. The hepatic dysfunction symptoms are highly variable, ranging from enlargement of the liver, or asymptomatic biochemical abnormalities, to overt cirrhosis or acute hepatic failure[3].

Neurologic or neuropsychiatric manifestations of WD typically present later than liver disease, most commonly in the third decade of life, but can also be present in childhood. There are a range of presenting neurologic abnormalities which are initially present in 40350% of patients[4]. They include Parkinson9s-like akinetic-rigid syndrome, pseudosclerosis with tremor, ataxia, and dystonic syndrome. Neuropsychiatric abnormalities can also develop, most frequently in patients who also display neurological dysfunction, thus reflecting their Central Nervous System origin. These most commonly include personality changes and mood disturbances, particularly depression, but they may also manifest as impulsiveness, disinhibition, paranoia, or poor school performance.

Dietary control of copper intake is not sufficient and pharmacological treatments are therefore needed.

1.2 Current Treatment

Treatment is primarily aimed at removing copper that has accumulated in organ tissue and preventing the accumulation of further copper. This is achieved with potent chelator therapies which extract copper from the tissues and promote copper excretion. Traditionally, D-penicillamine has been the first line chelation agent. However, approximately a third of patients cannot tolerate long-term D-penicillamine treatment due to side effects[2].

Trientine, also referred to as triethylene tetramine (TETA), is an organic compound with the formula $[CH_2NHCH_2CH_2NH_2]_2$. In Europe, trientine has been predominantly used as a second-line chelator, for those unable to tolerate D-penicillamine. The use of Trientine for treating WD is well established and supported by extensive literature. The primary mechanism of action of trientine is the formation of a stable molecular complex with copper, which is then excreted in the urine. Trientine prevents copper accumulation in tissues by increasing the urinary excretion of copper and reducing copper levels in the liver and kidney[5,6]. In addition, trientine may bind to copper in the gut and reduce copper absorption.

Most orally ingested trientine is not absorbed but excreted unchanged in the faeces. The 5 to 18% that is systematically absorbed in humans is reported to display extensive tissue distribution, with high levels of distribution to the liver and kidneys[7,8]. Systemically absorbed trientine is extensively

metabolised, with acetylation into two major metabolites, N1-acetyltriethylenetetramine (MAT) and N1,N10-diacetyltriethylenetetramine (DAT). Trientine and its metabolites are rapidly excreted in the urine, although low levels of trientine can still be detected in the plasma after 20 hours[9], there is no evidence of accumulation. Evidence suggests that steady state concentrations are achieved with regular administration of trientine at recommended dose levels[10].

Trientine tetrahydrochloride (TETA 4HCl) is a formulation of trientine that was developed to be stable at room temperature, unlike the previous Trientine compound, Trientine hydrochloride (TETA 2HCL). A TETA 4HCL tablet, containing 150 mg of trientine base, has previously received marketing authorisation in the EU, UK, and further afield under the tradename of Cuprior®. Cuprior® is licensed for the treatment of WD in adults, adolescents, and children aged g 5 years intolerant to D-penicillamine therapy. Additionally, TETA 4HCl has received marketing authorisation in USA in 2022 under the tradename Cuvrior™ for a converse indication, the treatment of adults with stable WD tolerant to D-penicillamine.

1.3 Study Rationale

As in all chronic diseases, medication compliance, adherence and persistence are key parameters to improve prognosis, but unfortunately are often suboptimal. In WD, available data concerning medication adherence and persistence are limited. However, periods of non-adherence and low persistence have been shown to be associated with significant progression of disease[11,12,13].

All current treatments for WD require a multiple daily dosing posology, combined with the requirement to administer each dose on an empty stomach, at least one hour before meals or two hours after meals and at least one hour apart from any other medicinal product, food, or milk. These challenging requirements, along with pill/capsule burden (number/size) have been identified as the key obstacles to adherence for WD patients[14]. In a prospective study[14], 139 Wilson disease patients were assessed for adherence using the Morisky medication adherence scale. The study revealed a significant low rate of adherence in the majority of patients, with only 21% maintaining high adherence and with 47% and 32% of patients exhibiting medium and low treatment adherence, respectively.

To address the challenge of adherence, compliance, and persistence in WD, Orphalan SA have developed a new TETA 4HCL tablet formulation, which contains 300mg trientine base, intended for once daily dose administration. This is a hybrid medicine based on the reference medicine, Cuprior® (TETA 4HCL)[15]. This new hybrid medicine contains twice as much trientine base per tablet compared to Cuprior®, and will aid once daily dosing. The formulations are compared in Table 1.

Table 1: Drug Formulation Properties

	Cuprior®	New Formulation
Appearance	Yellow film coated scored tablet	Yellow film coated scored tablet
Active compound	TETA 4HCL	TETA 4HCL
Trientine base, mg	150	300
Excipients	Mannitol Colloidal anhydrous silica Glycerol dibehenate	Mannitol Colloidal anhydrous silica Glycerol dibehenate Magnesium-stearate
Film-coating	Opadry AMB II 88A120000 Yellow	Opadry AMB II 88A120000 Yellow

This study aims to assess the pharmacokinetics and safety and tolerability of a total daily dose of 900mg of TETA 4HCL, comparing the new once daily TETA 4HCL formulation (3x300mg trientine base tablets, OD) with the current marketed Cuprior® formulation, administered 3x150mg trientine base tablets administered twice a day in healthy adult male and female participants. Current recommended

initial doses of TETA 4HCl in Europe are between 450mg and 975mg of trientine base, administered in two to four divided doses and titrated to clinical response. Some literature has supported a once daily dosing regimen but there is a paucity of pharmacokinetic data to support this.

Exploring the PK profile of once daily TETA 4HCL administration will be used to support a once daily dosing regimen in patients. This reduction in treatment burden will increase overall therapeutic compliance, and ultimately lead to better clinical outcomes for patients with WD.

2. TRIAL OBJECTIVES AND OUTCOMES

2.1 Objectives

Primary		
Objectives	**Endpoints**	**Statistical analyses**
To evaluate and compare the plasma concentration of TETA 4HCL (active moiety, TETA) and its two main metabolites (N1-acetyltriethylenetetramine (MAT) and N1, N10-diacetyltriethylenetetramine (DAT)) following administration of a total daily dose of 900mg TETA 4HCL, comparing the new once daily TETA 4HCL formulation (3x300mg trientine base tablets, OD) with the current marketed Cuprior® formulation, (3x150mg trientine base tablets, BD) in healthy adult male and female participants.	PK parameters derived by non-compartmental methods including: area under the plasma concentration-time curve from time zero to last detectable plasma concentration (AUC_{0-t}), area under the plasma concentration-time curve from time zero to 24 hours plasma concentration (AUC_{0-24}), area under the plasma concentration-time curve from time zero extrapolated to infinite ($AUC_{0-¥}$), maximum observed plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), apparent total plasma clearance (CL/F), apparent volume of distribution during the terminal phase (V_z/F), terminal elimination rate constant ($»_z$), and terminal elimination half-life ($t_{1/2}$).	″ Plasma concentrations will be listed and summarised by time point. Individual and arithmetical mean plasma concentration vs time curves for each analyte which includes all treatments will be produced on both linear/linear and log10/linear scales. ″ The PK parameters will be listed for each participant and summarized for each treatment group using descriptive statistics (N - the number of participants, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum). ″ For comparability of the two products the geometric mean, ratio of geometric means, confidence intervals (CI) including their logarithmic transformation together with the coefficient of variation for the within-participant variability will be summarised. In case a gender effect is noticeable, gender will be added as an additional term in the PK model, and all PK analyses will be presented per gender as well.
Secondary		
Objectives	**Endpoints**	**Statistical analyses**
To compare the safety and tolerability of the two TETA 4HCL tablet formulations.	″ The incidence, severity, and relationship of Treatment-Emergent Adverse Events (TEAEs). ″ Proportion of participants with clinically significant changes in laboratory safety tests (haematology, biochemistry, coagulation, and urinalysis).	″ Adverse Events (AE), Serious Adverse Events (SAEs), vital signs, ECG parameters, and clinical laboratory data will be listed and summarised using descriptive statistics. ″ All AEs will be summarised and listed by using System Organ Class (SOC) and Preferred Term (PT) assigned to the event using Medical

	″ Proportion of participants with morphological and/or rhythm abnormalities on electrocardiogram (ECG). ″ Proportion of participants with clinically significant changes in vital signs (systolic blood pressure, diastolic blood pressure and pulse rate).	Dictionary for Regulatory Activities (MedDRA). Furthermore, these events will be summarised by the maximum intensity. The number of participants who experienced drug-related AEs will also be summarised. Any SAEs and/or AEs that led to withdrawal will be summarised and listed.

3. TRIAL DESIGN

3.1 Overall trial design

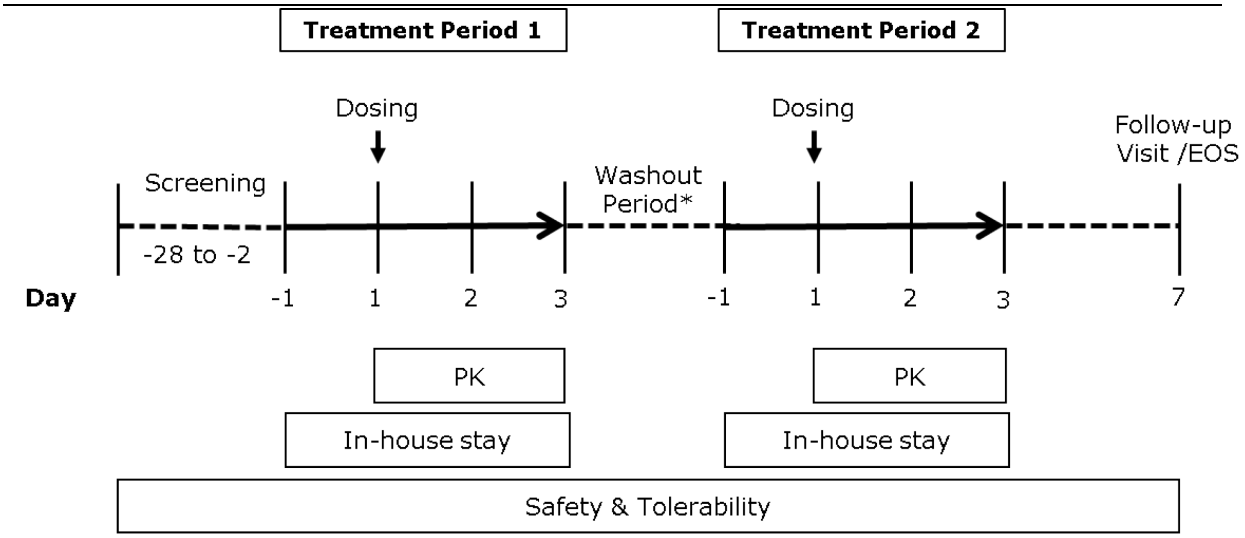
This is a phase I, single centre, randomised, interventional, open-label, cross-over study to evaluate the PK, safety, and tolerability of a total daily dose of 900mg of TETA 4HCL, comparing the new once daily TETA 4HCL formulation (3x300mg trientine base tablets, OD) with the current marketed Cuprior® formulation (3x150mg trientine base tablets, BD) in adult healthy male and female participants.

3.1.1 Choice of participants and dose rationale

This study will enrol 26 healthy participants composed of both male and female participants who have met all the eligibility criteria. 26 participants will be enrolled to ensure 24 participants complete the study. Enrolment will target a balanced gender split, with a maximal 60:40 gender split accepted. All women of childbearing potential (WOCBP) will undergo a serum beta human chorionic gonadotropin (^{3}-HCG) pregnancy test at screening and a urinary pregnancy test on D-1 of the treatment periods. Women that are breastfeeding will be excluded from participating in the study. Post-menopausal females will undergo FSH testing at screening to confirm menopausal status.

A total daily dose of 900mg trientine base has been selected as this aligns with the approved Cuprior® indication of 450-975mg initial total daily doses. A previous PK study, in which healthy participants received high and low doses of TETA formulations, observed no change in the safety profile with increasing dose level[16].

The overall trial design is depicted in Figure 1.



* At least 5 days and a maximum of 10 days between treatment period study drug administrations

Figure 1: Study flow chart

The study will be conducted at a single site in the UK. Each participant will receive verbal and written information before signing the Informed Consent Form (ICF) prior to any screening procedures taking place. Participants will undergo screening (D-28 to D-2) to assess eligibility. Enrolled participants will undertake two inpatient treatment periods, separated by an intermediate washout period (at least 5 days and a maximum of 10 days between study drug administration). At each treatment period, participants will be admitted to the unit on D-1 and will be discharged on the morning of D3. On D1 of the first treatment period, participants will be randomised (1:1) to one of two treatment sequences, as described in Table 2. Study drug administration will occur on D1.

Table 2: Treatment Sequences

Treatment Sequence	**Period 1**	**Period 2**
Sequence 1	Treatment A	Treatment B
Sequence 2	Treatment B	Treatment A

Treatment A: 900mg TETA 4HCl, new once daily formulation (3x300mg trientine base tablets as a single AM dose).

Treatment B: 900mg TETA 4HCl, Marketed Cuprior® formulation (6 x150mg trientine base tablets in two equally divided doses (450mg doses 8 hours apart).

Participants will return to the unit for an outpatient, end of study follow-up visit on D7 of treatment period 2. All assessments performed during the study are detailed in the study schedule of assessments (Table 3 & 4). The total duration of the study (including the screening period) is approximately 7 weeks.

Table 3: Schedule of Assessments

Period	Screening	Treatment period 1				Treatment period 2[a]				Follow-up Visit/ End of Study
Procedure (allowable window)	**D-28 to D-2**	**D-1**	**D1**	**D2**	**D3**	**D-1**	**D1**	**D2**	**D3**	**D7 (+/-2d)**
Informed consent [b]	X									
Demographic data	X									
Body weight/height/BMI [c]	X	X				X				X
12-lead ECG [d]	X	X				X				X
Vital signs [e]	X	X	X	X		X	X	X		X
Medical/Surgical history and prior medication	X									
Smoking history	X									
Physical examination [f]	X	X		X		X		X		X
Urine drugs of abuse screen	X	X				X				
Breath alcohol test	X	X				X				
Haematology/Biochemistry /Coagulation [g]	X	X		X		X		X		X
Serology (HIV 1 & 2, Hep B & C)	X									
Urinalysis	X	X		X		X		X		X
Urine Cotinine test	X									
Pregnancy testing (females) [h]	X	X				X				X
FSH [i]	X									
Inclusion/Exclusion criteria [j]	X	X								
Check-in		X				X				
Check-out					X				X	
Non-residential visit	X									X
Randomisation			X							
Study drug administration [a,k]			X				X			
Meals [l]		X	X	X	X	X	X	X	X	
PK blood sampling [m]			X	X	X		X	X	X	
COVID-19/Influenza testing [n]	X	X	X	X	X	X	X	X	X	X
AE recording/concomitant medication [o]	Throughout									

a. The study drug administration between treatment periods will be at least 5 days and a maximum of 10 days (Washout period).
b. Study procedures will not occur until informed consent is completed. Participants will not be reconsented if they are rescreening for the trial.
c. Height will be measured at Screening only.
d. 12-lead ECGs (triplicate) will be measured after at least 10 minutes rest in supine position.

e. Vital signs (respiratory rate, tympanic temperature, blood pressure and heart rate) will be measured after at least 5 minutes rest in supine position. D1 vital signs will be performed 2 hours prior to AM dosing and at H6.

f. Full physical examination will be carried out at screening and EOS visits, symptom directed physical examination will be performed at other time-points.

g. D2 samples to be taken at H24.

h. Serum pregnancy test will be performed at screening. A urine pregnancy test will be performed at all other time-points.

i. FSH test will only be performed at screening on post-menopausal women to confirm post-menopausal status.

j. Inclusion and exclusion criteria will be assessed at screening and treatment period 1 D-1. Participants will be assessed on treatment period 2 D-1 to ensure the participant has not had any significant changes to their health with may preclude further dosing.

k. The study drug will be administered on Day 1 with 240ml water. The order of the treatment sequence is determined by the randomisation schedule.

l. Detailed D1 timings are provided in Table 4.

m. Timings of PK samples are detailed on Table 4.

n. COVID-19/influenza point of care testing will be carried out as per the site9s latest COVID-19 Infection Control Guidelines and pre-entry algorithms.

o. AEs and concomitant medications will be collected at each visit from the time of consent until the end of study visit, and daily during study residency.

Table 4: Schedule of assessments (cont.)

Timepoint		Treatment A		Treatment B		Generic	
Study Day	**Time (h)**	**Dose administration**	**PK sampling (plasma)**	**Dose administration**	**PK sampling (plasma)**	**Meals[a]**	**Fasting[b]**
D1	Pre-dose		x		x		x
	0	x		x			x
	0.5		x		x		x
	1		x		x	x	
	1.25		x		x		
	1.5		x		x		
	2		x		x		
	3		x		x		
	4		x		x		
	5		x		x	x	
	6		x		x		x
	8		x	x	x[c]		x
	8.5				x		x
	9				x		
	9.25				x		
	9.5				x		
	10				x		
	11				x		
	12		x		x	x	
	13				x		
	14				x	x	
	16		x		x		
	20				x		
D2	24		x		x		
	36		x		x		
D3	48		x		x		

a. D1 meals for both treatment periods will be identical and will follow the scheduled times above. D2/D3 meals will follow the standard timings of the research unit and are not described in this table.

b. Participants will be fasted overnight (minimum 8 hours) prior to H0 dose administration. Participants will be fasted for at least 2 hours prior to H8. Participants will be nil by mouth for 1 hour following all study drug administrations.

c. H8 PK sample must be taken just before (within 10mins) the second treatment B dose.

3.2 Adaptive Protocol Features

This trial incorporates adaptive protocol features which may be implemented at the discretion of the investigator. These adaptive protocol features and their limits are described Table 5.

Table 5: Adaptive Protocol Features

Additional safety assessments	1. Additional safety assessments may be performed on an individual participant if it is considered clinically necessary by the Site Investigator on a case-by-case basis. 2. Additional safety assessments may refer to either: a. an increased number of the same safety assessments planned in the existing Schedule of Assessments. b. additional parameters (specific tests) on assessments already scheduled e.g., troponin tests on safety blood samples. c. additional safety tests requiring additional blood/urine sample collections or other clinical procedures e.g., ultrasound scans. 3. Specialist referrals (e.g., to a cardiologist) may be made (and may include all relevant assessments and investigations) if it is considered clinically necessary and agreed by the Principal Investigator (PI) and Sponsor (unless considered an acute emergency by site PI), on a case-by-case basis.	I. The maximum blood volume for scheduled assessments is given in Section 8.10. This maximum may only be exceeded if additional unscheduled blood samples/ investigations are performed as necessary to ensure the safety of the individual participant. II. A maximum number of specialist referrals for individuals will be determined on a case-by-case basis and cannot be pre-defined as investigations will be performed as necessary to ensure the safety of the individual participants.
Screening	1. Screening assessments performed (prior to signing the ICF for this study) at Richmond Pharmacology on participants screened (but not randomized) for another study or screening for a generic protocol can be used for this study to avoid unnecessary tests/repeats.	I. The assessments must meet protocol criteria (e.g., the method to be used). II. The assessments must be performed within the protocol defined Screening window (Day -28 to -2).
Replacement of withdrawn participants	1. Withdrawn participants can be replaced at the discretion of the Sponsor and the Investigator. The eligible replacement participant(s) will be enrolled into the same treatment sequence as the original participant(s) and will complete both treatment periods and the end of study visit as per the schedule of assessments. Evaluable data from the withdrawn participant will be included in the analysis as appropriate.	I. Participants will only be replaced to fulfil a total of 24 participants completing the study. II. Trial stopping rules apply to all participants.

4. RISK MANAGEMENT

4.1 Potential Benefits

TETA 4HCL will be given to healthy participants for research purposes only. It is not anticipated that participants will receive any medical benefit apart from a general health examination.

4.2 Potential Risks

4.2.1 TETA 4HCL

Potential risks are expected to be similar to the reference medicine, Cuprior® (150mg trientine base per tablet). The Cuprior®SmPC reports the following adverse reactions: iron deficiency anaemia, sideroblastic anaemia, nausea, duodenitis, colitis (including severe colitis), skin rash, pruritus, erythema and urticaria. Potential risks will be closely monitored for as part of the safety evaluations being performed in this trial. The risk mitigation measures are summarised in Table 6.

Table 6: Potential risks and mitigation strategies per target system

Target System	Effect	Risk Mitigation
Haematological system	1) Iron Deficiency Anaemia 2) Sideroblastic Anaemia	1) Participants with clinically significant haematological pathology or laboratory abnormalities (in the opinion of the investigator) will be excluded from participation. 2) Safety bloods, including a full blood count, will be taken at regular intervals highlighted in the schedule of assessments. 3) Adverse events will be collected throughout the study.
Gastrointestinal system	1) Nausea 2) Duodenitis 3) Colitis	1) Participants with clinically significant gastrointestinal pathology (in the opinion of the investigator) will be excluded from participation. 2) Safety bloods tests will be taken at regular intervals highlighted in the schedule of assessments. 3) Adverse events will be collected throughout the study.
Cutaneous and Mucosal	1) Skin rashes 2) Pruritis 3) Erythema 4) Urticaria	1) Participants with clinically significant dermatological pathology (in the opinion of the investigator) will be excluded from participation. 2) Physical examination will be performed at Screening and post dose to monitor for cutaneous and mucosal reactions. 3) Adverse events will be collected throughout the study.

4.2.1.1 Summary

TETA 4HCL has a relatively well-established safety profile, as Cuprior® received marketing authorisation in 2017 and previous formulations of trientine have been used in the UK since 1985. Some of the known side effects (iron deficiency anaemia, sideroblastic anaemia) relate to chronic dosing and the long-term chelation of other metals (i.e., iron, zinc etc.). This is unlikely to occur when healthy participants receive single doses. In applying the above risk management strategies, the overall risk to participants in this trial is considered low.

4.2.2 Investigator Site Facilities and Personnel

This trial will be conducted in a specialised early phase Clinical Pharmacology Unit with onsite resuscitation equipment and medication, in addition to access to an acute hospital with Critical Care facilities. The trial is conducted by an experienced PI and well trained medical and technical staff with ample experience in the conduct of early phase clinical trials.

The trial is designed to closely monitor, treat, and communicate potential expected adverse reactions as well as potential unexpected adverse events.

5. DECISION-MAKING RULES AND LIMITS

5.1 Stopping Rules

If either of the following scenarios occur, study enrolment and dosing will be suspended immediately:

- If g1 participant experiences a serious AE that is considered at least possibly related to the IP.
- If g2 participants experience severe non-serious AEs that are considered at least possibly related to the IP, independent of within or not within the same system organ class (SOC).
- If the Sponsor or Investigator considers there to be an unfavourable benefit-risk ratio based on emerging safety data.

If, following consultation between the PI and the Sponsor, it is considered appropriate to restart enrolment/dosing in the remaining participants, a substantial amendment will be submitted to the Medicines and Healthcare Products Regulatory Agency (MHRA) for restarting the study.

5.1.1 Individual Stopping Rules

In addition to the stopping rules described in 5.1, any participant who has received a dose of IMP and is scheduled to receive further doses of IMP, will not receive any further doses of IMP if either of the following occur:

- If the participant experiences a severe non-serious AE that is considered at least possibly related to the IP.
- If the investigator or sponsor considers there to be any other factor which may jeopardise the safety of the individual participant.

6. SELECTION AND WITHDRAWAL OF PARTICIPANTS

6.1 Replacement participants

Additional participants may be recruited to replace any withdrawn participants, in order to ensure that 24 participants complete the study. Participant replacement is detailed in the Adaptive Protocol Features (Table 5).

6.2 Inclusion Criteria

Participants must meet all the following criteria to be eligible for enrolment in this study:

1. Ability to provide written, personally signed, and dated informed consent to participate in the trial, in accordance with the ICH Good Clinical Practice (GCP) Guideline E6 (R2) (2016) and applicable regulations, before any study-specific procedure is performed.
2. Male or female aged g18 to f40 years at the date of signing informed consent which is defined as the beginning of the Screening Period. This inclusion criterion will only be assessed at the Screening Visit.
3. Body weight g 50 kg and body mass index (BMI) of 18.0 to 25.0 (inclusive) kg/m^2 at Screening.
4. Healthy as defined by:

a. The absence of clinically significant illness and surgery within twelve weeks prior to dosing.

b. The absence of clinically significant history of neurological, endocrine, cardiovascular, respiratory, haematological, immunological, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease.

5. Satisfactory medical assessment with no clinically significant or relevant abnormalities as determined by medical history, physical examination, vital signs, 12-lead ECG, and clinical laboratory evaluation (haematology, biochemistry, coagulation, and urinalysis) that is reasonably likely to interfere with the participant's participation in or ability to complete the trial as assessed by the Investigator.
6. Participants must agree to adhere to a contraceptive method defined in Appendix 1.
7. An understanding, ability, and willingness to fully comply with trial procedures and restrictions.

6.3 Exclusion Criteria

Participants will be excluded from enrolment in this study if they meet any of the following criteria:

1. Any significant current or recurrent disease (e.g., cardiovascular, haematological, dermatological, neurological, endocrine, immunological, renal, hepatic, respiratory, or gastrointestinal or other conditions).
2. Acute significant disease or illness (e.g., vomiting, fever, diarrhoea) within 7 days before study Day 1 of Treatment period 1.
3. Any clinically significant deviations in haematology, biochemistry, or coagulation parameters at screening. Repeat testing is permitted at the discretion of the investigator.
4. An estimated Glomerular Filtration Rate (eGFR) <60 ml/min/1.73m^2 at screening.
5. Positive test result for alcohol at Screening or Day -1 of each treatment period.
6. History of drug abuse or excessive alcohol intake. Excessive alcohol intake is defined as regular consumption of more than 14 units of alcohol per week.
7. Positive test result for drugs of abuse at Screening or Day -1 of each treatment period.
8. Positive test for Hepatitis B surface antigen (HBsAg), Hepatitis C antibody (HCV Ab), or human immunodeficiency virus antibody (HIV Ab) at Screening.
9. Female participants who are pregnant (including a positive pregnancy test at Screening and on Day-1 of each Period) or breastfeeding.
10. History or regular use of tobacco in any form (e.g., smoking or chewing) or other nicotine-containing products in any form (e.g., gum, patch, electronic cigarettes) within 6 months prior to Screening.
11. Positive urine cotinine level at screening.
12. Treatment with an investigational drug within 90 days or 5 half-lives (whichever is the longer) or exposure to more than 3 investigational drugs within 12 months of first study drug administration.
13. Has used any of the following:

    Prescription medication (excluding female hormonal contraception or hormone replacement therapy) within 30 days or 5 half-lives (whichever is longer) prior to first study drug administration.

OR

Over the counter (OTC) medication (including multivitamin, herbal, or homeopathic preparations; (Paracetamol use f2g per day is permitted)) during the 14 days or 5 half-lives of the drug (whichever is longer) before first study drug administration.

14. History of sensitivity/allergy to the study medications or components thereof (mannitol, colloidal anhydrous silica, glycerol dibehenate or magnesium-stearate).
15. Donation or loss of 450 mL or more of blood or plasma within 16 weeks prior to first trial medication administration or intention to donate blood in the 16 weeks after completing the trial.
16. An inability to follow a standardised diet and meal schedule or inability to fast, as required during the trial.
17. Participants deemed to have difficult veins for cannulation/blood draws.

6.4 Participant Restrictions

Participants will have to comply with the restrictions described in Table 7.

Table 7: Participant Restrictions

Items participants must not consume or do:	When participants must stop:	When participants can re-start:
Tobacco in any form (e.g., smoking or chewing) or other nicotine-containing products in any form (e.g., gum, patch, electronic cigarettes)	From six months prior to screening.	After trial completion.
Meals/snacks/water	During the inpatient stays, only the meals/drinks provided by the trial personnel will be allowed. Standard meals will be provided at set times as stated in the trial plan, and meals should be completed each time.	After discharge from the unit.
Caffeine-containing or Xanthine-containing products	48 hours before the planned trial drug administrations and follow-up visit.	After trial completion.
Energy drinks or drinks containing taurine, glucuronolactone (e.g., Red Bull)	48 hours before the planned first trial drug administration and each out-patient/ follow-up visit.	After trial completion.
Alcohol	48 hours before the planned trial drug administrations and follow-up visit.	After trial completion.
Strenuous physical activity. (Participants should not start new	48 hours before admissions and follow-up visit.	After trial completion.

Items participants must not consume or do:	When participants must stop:	When participants can re-start:
physical training activities during the trial until trial completion)		
Any prescription medication (excluding female hormonal contraception or hormone replacement therapy)	30 days or 5 half-lives (whichever is longer) before the planned first trial drug administration.	After trial completion. N.B: If participants have a medical need to take any medication or have any medications prescribed to them by a doctor, they should follow the medical advice but inform the Investigator as soon as possible afterwards. Participants should be informed not to stop taking any medication that has been prescribed by their GP or other doctor.
Any over the counter (OTC) medication (Paracetamol use f2g per day is permitted).	14 days or 5 half-lives (whichever is longer) before the planned first trial drug administration.	
Blood and plasma donation	16 weeks before the planned first trial drug administration.	16 weeks after trial completion.

6.5 Criteria for withdrawal

The investigator or designee may withdraw a participant from the trial if the participant:

- Is in violation of the protocol, which may jeopardize the trial results or represent a risk to the participant
- Has an AE warranting withdrawal
- Becomes pregnant
- Use of/need for a prohibited medication which in the opinion of the sponsor or investigator may jeopardize the trial results or represent a risk to the participant
- Requests to be withdrawn from the trial (participant withdrawal of consent)
- Is found to be considerably non-compliant with the protocol-required dosing visits
- In the investigator9s opinion, is unable to continue trial participation
- Is withdrawn from the trial upon the request of sponsor, including if sponsor terminates the trial.

6.5.1 Handling of Withdrawals

In the event a participant withdraws or is withdrawn from the trial, the investigator will inform the sponsor immediately. If there is a medical reason for withdrawal, the participant will remain under the supervision of the investigator for protocol-specified safety follow up procedures.

Should any of the participants be withdrawn from the trial (by the investigator and/or sponsor) after being dosed, all the relevant assessments in relation to last dose should be completed as per protocol. The investigator and/or sponsor may decide to perform additional (or less) assessments in accordance with Table 53 Adaptive Protocol Features.

Should a participant withdraw themselves from the trial, every effort should be made to conduct a complete Early Termination (ET) visit at an appropriate time-point. The procedures required for the 8Last Visit9 should be performed at this visit. The investigator and/or sponsor may request additional (or less) assessments in accordance with Table 5**3** Adaptive Protocol Features, with the participant9s

agreement.

A participant who fails to return for final evaluation will be contacted by the site in an attempt to have the participant comply with the protocol in accordance with the site SOPs.

When a participant withdraws from the trial, the primary reason for discontinuation must be recorded in the appropriate section of the case report form (CRF).

7. TRIAL IMP AND CONCOMITANT TREATMENTS

The trial treatments are categorised and described below.

An **Investigational Medicinal Product (IMP)** is defined as a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorisation but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form.

- The term <**test IMP**= is used to indicate only the experimental product under trial or development. Note: This term is more specific than <investigational medicinal product= which includes comparators and placebos.
- The term <**reference IMP**= is used to indicate any medicinal product being used as a comparator or reference substance (including placebo). Note: This term is more specific than <investigational medicinal product= which includes the experimental product and placebos.

7.1 Investigational Medicinal Products (IMPs):

The following IMPs will be used in this trial:

- Cuprior® 150 mg film-coated tablets [Reference IMP]
- TETA 4HCL new once daily formulation 300mg tablets [Test IMP]

Bulk Test IMP will be supplied and QP certified by Orphalan SA or their contractor. Reference IMP will be obtained by Richmond Pharmacology through an approved Richmond Pharmacology supplier. Prior to being used on the clinical trial, the IMPs will be re-packaged and prepared by Richmond Pharmacology9s clinical trials pharmacy. The packaged and prepared individual participant doses will be certified and dispensed by the site9s pharmacy staff.

7.1.1 Labelling of IMPs

The labelling of the trial drugs will be in compliance with Good Manufacturing Practice (GMP) specifications, as described in The Rules Governing Medicinal Products in the European Union, Volume 4, Annex 13, Investigational Medicinal Products, and any other or local applicable regulations.

Sample label(s) will be submitted to the UK health authorities according to the submission requirements.

7.1.2 Drug administration

The two TETA 4HCL tablet (Oral) formulations tested in this study will be administered to participants with 240 mL of water.

Study drug administration will be performed by a Research Physician and the details of dosing will be recorded in the CRF. The dosing will be verified by another member of the investigator9s staff. Detailed instructions for dose administration will be included in the SOM.

7.1.3 Storage of IMPs

IMP will be stored in accordance with the labelling instructions as defined in the IMPD. The IMPs will be stored securely in a temperature-controlled pharmacy with authorised access only.

7.1.4 Drug Accountability

The designated pharmacy staff at the clinical trial site will maintain accurate records of receipt and the condition of all trial drugs, including dates of receipt. In addition, accurate records will be kept by the pharmacy staff of when and how much trial drug is dispensed and used by each participant in the trial. Any reason for departure from the protocol dispensing regimen must also be recorded.

Drug accountability records and inventory will be available for verification by the sponsor or designee. At the completion of the trial, there will be a final reconciliation of all trial drugs.

Trial drug must not be used for any purpose other than the present trial. Remaining trial drug will be returned to the sponsor or its agent or its destruction arranged by the clinical trial site according to applicable regulations and only after receipt of written authorization from the sponsor.

7.2 Treatment Allocation

7.2.1 Participant Randomisation

All participants in this trial will be assigned to a treatment regimen according to a randomisation schedule generated by a statistician. Eligible participants will be randomly assigned on D1 to one of the two treatment sequences (1:1 ratio) detailed in Table 2.

Details regarding the unique screening and participant number allocation will be included in the SOM.

8. TRIAL METHODOLOGY

8.1 Order of Procedures

Order of Procedures / Priorities:

When multiple assessments occur at a specific timepoint, PK blood sampling takes priority (takes precedence over ECG and vital sign measurements) and is to be scheduled at the exact time point defined in the Schedule of Assessments. During the In-house Period, the order of procedures should be ECG, Vital Signs, Blood Samples including PK, and Meal. Should there be any delay to procedures, PK sample timings should be prioritized above the order of procedures.

Scheduling time windows:

The permitted time windows are set out in the Data Handling Protocol for scheduling time windows (DHP STW) and factored into the design of the Master Treatment Schedule (MTS).

Protocol deviations:

Where the time of clinical assessment deviates from the scheduling time window in the DHP STW, this will be considered a protocol deviation. These deviations will be identified, classified (minor, major, critical), and managed in accordance with the DHP STW.

8.2 Medical History

All clinically significant medical history (including any significant surgical history) must be reviewed and recorded on the CRF for each participant. Each participant9s full medical history will be obtained through direct questioning, medical assessment at Screening and GP Health Care Form review.

Any changes to medical history occurring during the Screening Period and prior to first study drug administration on Day 1 will be documented.

8.3 Meals

Dosing day (D1) meals will be identical across treatment periods. Meals will be provided at the timepoints dictated in Table 3 & 4 . Meals will consist of the following:

- Breakfast (1 hour post AM dose)
- Lunch (5 hours post AM dose)
- Dinner (12 hours post AM dose)
- Snack (14 hours post AM dose)

Meals for the non-dosing inpatient days will be served as below, following the standard timings of the research unit:

- Breakfast (following the morning procedures)
- Lunch
- Dinner
- Snack

8.4 Clinical Laboratory Assessments

Laboratory parameters to be measured are presented in Table 8.

Table 8: Laboratory parameters

Haematology	Biochemistry	Urinalysis
″ Platelets	″ Aspartate aminotransferase (AST)	″ Leukocytes
″ Haemoglobin (Hb)	″ Alanine aminotransferase (ALT)	″ Nitrite
″ Haematocrit	″ Alkaline phosphatase (ALP)	″ Urobilinogen
″ White blood cells	″ Urea	″ Protein
″ Neutrophils cell count	″ Gamma GT	″ pH
″ Eosinophils cell count	″ Total bilirubin	″ Blood
″ Basophils cell count	″ Direct bilirubin	″ Specific gravity
″ Lymphocytes cell count	″ Creatinine	″ Ketones
″ Monocytes cell count	″ eGFR*	″ Bilirubin
″ Red blood cells	″ Total Serum Proteins	″ Glucose
″ Mean cell haemoglobin (MCH)	″ Albumin	″ Urine microscopy**
″ Mean corpuscular haemoglobin concentration (MCHC)	″ Creatine kinase	″ Urine pregnancy test***
″ Mean corpuscular volume (MCV)	″ Sodium	
″ HbA1c*	″ Potassium	
	″ Calcium	
	″ Corrected calcium	
	″ Magnesium	
	″ Chloride	
	″ Bicarbonate	
	″ Amylase	
	″ Total cholesterol	
	″ Triglycerides	
	″ Serum pregnancy test***	
	″ FSH*/***	

Coagulation
″ aPTT
″ PT (or INR in patient studies)
″ Fibrinogen
″ INR

Serology*
″ Hepatitis B surface antigen (HBsAg)
″ Hepatitis B core antibody (anti-HBC IgG + IgM, if IgG positive)
″ Hepatitis C antibody (anti-HCV)
″ HIV I and II antibodies

Urine Screen for Drugs of Abuse
″ Benzodiazepines
″ Opiates
″ Amphetamines
″ Methadone
″ Cocaine
″ Cannabinoids
″ Barbiturates
″ Cotinine*

* Only measured at screening.
**Only if urinalysis demonstrates one of the following: positive nitrites, positive leucocytes, >trace protein or blood (unless female and menstruating,). Microscopy may be requested at the discretion of the investigator outside of the aforementioned parameters.
*** Pregnancy test for pre-menopausal women only; FSH for post-menopausal women only.

8.4.1 Haematology, Biochemistry, and Coagulation

Blood samples for determination of biochemistry, haematology and coagulation parameters will be taken at the times given in the Schedule of Assessments (Table 3 & 4). The date and time of collection will be recorded on the appropriate CRF pages. The analyses will be done using routine methods. eGFR will be calculated using the CKD-EPI (2009) equation. Further details will be described in the SOM.

Laboratory values outside the reference limits, which are suspected to be of any clinical significance, will be repeated. Participants in whom the suspected clinical significance is confirmed on repeated sampling will either not be included or, if already included, may be withdrawn from further participation in the trial and/or followed-up until normalisation or for as long as the Investigator considers necessary.

8.4.2 Serology

Serology will be performed at Screening as detailed in the Schedule of Assessments (Table 3 & 4). At the screening visit all participants will be tested for the parameters listed in Table 8.

If a participant is found to be confirmed positive in any of these tests, they will be referred for further examination/treatment and will not be included in the trial, except for participants with a confirmed positive anti-HBc IgG and negative anti-HBc IgM and negative HBsAg, indicative of natural immunity due to a past infection without active chronic or acute infection.

8.4.3 Pregnancy/FSH testing

Serum pregnancy tests will be performed on WOCBP at screening. A urine pregnancy test will be performed at all other time-points, as per the schedule of assessments. Additional pregnancy tests may be performed if pregnancy is suspected. Participants who are pregnant will not be eligible for study participation.

FSH test will only be performed at screening on post-menopausal women to confirm post-menopausal status.

8.4.4 Urinalysis

Urine samples for determination of urinalysis parameters will be taken at the times given in the Schedule of assessments. If deemed necessary, based on a clinically significant abnormal test, microscopic examination of urine will be performed.

8.4.5 Drugs of Abuse

Urine will be tested for the drugs of abuse as described in the Schedule of assessments. If a participant fails the drug abuse screen, they will be excluded from the trial. A repeat drug screen can be done where methodological reasons are believed to have led to a false positive. If participants are suspected to be positive due to medication e.g., flu/cold remedies, they may undergo a repeat drug screen. A urine Cotinine will also be assessed at screening.

8.5 Alcohol Breath Test

An alcohol breath test will be done using an alcometer as described in the Schedule of Assessments. If a participant tests positive to the test they will be excluded from the trial.

8.6 Vital signs (blood pressure, pulse rate, respiratory rate, and tympanic temperature)

Vital signs will be measured at the time points as detailed in the Schedule of Assessments. Blood pressure and pulse rate will be measured in supine position after the participant has rested comfortably for at least 5 minutes using automated blood pressure monitors. Respiratory rate will be measured by manual counting for one minute. Temperature will be measured using tympanic thermometers. Detailed methodology will be provided in the SOM.

8.7 Electrocardiographic (ECG) Measurements

8.7.1 Recording of 12-lead ECGs

12-lead ECGs will be recorded at the time-points described in Schedule of assessments. 12-lead ECG recordings will be made after the participants have been resting in a supine position for at least 10

minutes. At each time point, the ECG will be recorded in triplicate, to reduce variance and improve the precision of measurement. Detailed methodology will be provided in the SOM.

8.8 Physical Examination, Height, and Weight

The physical examination performed at screening and at the end of trial visit will include an assessment of the following: general appearance, skin, eyes, ears, nose, neck, lymph nodes, throat, heart, lungs, abdomen, musculoskeletal system and extremities. A symptom-directed physical examination will be conducted at all other visits as needed based on reported signs and symptoms. The timings of the physical examinations are described in the Schedule of Assessments.

Height will be measured in centimetres and weight in kilograms. Measurements should be taken with participants wearing light clothing and without shoes using calibrated scales for all measurements. BMI will be calculated from the height and weight. Full details will be described in the SOM.

8.9 Pharmacokinetic Assessments

8.9.1 PK blood samples

For timing of individual samples refer to the Schedule of Assessments. The date and time of collection will be recorded on the appropriate CRF.

Plasma samples for determination of PK parameters of TETA 4HCL (active moiety, TETA) and its two main metabolites (N1-acetyltriethylenetetramine (MAT) and N1, N10-diacetyltriethylenetetramine (DAT)) will be analysed by QPS Netherlands B.V. using a validated method. Full details of the analytical methods used will be described in a separate bioanalytical report. All sample handling procedures, including the time of each sample collection, collection tube details, the time of placement into frozen storage (at the end of the sample workup), and the date of transfer or shipment of the samples to the responsible analyst will be documented in detail in the SOM and Laboratory Manual.

8.10 Volume of Blood Sampling

The maximum total blood volume collected from participants participating in this study will not exceed 350 mL overall. The NHS blood donation policy at the time of completion of this protocol (https://www.blood.co.uk/who-can-give-blood/) states that women can give 470 mL of blood every 16 weeks. Therefore, the 350 mL permitted in this study is approximately consistent with current clinical practice.

8.11 Adverse Events

The collection, evaluation and reporting of adverse events/reactions arising from this clinical trial will be performed in accordance with:

- Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use (8CT-39) (2011/C 172/01).
- International Conference on Harmonization (ICH) harmonised tripartite guideline on clinical safety data management: <Definitions and standards for expedited reporting= E2A.
- ICH harmonised tripartite guideline on development safety update report: E2F.
- ICH guideline E2F <Note for guidance on development safety update reports (DSUR)=.

All AEs will be collected from the signing of the ICF until the end of study visit. Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. If the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and the Investigator considers the event to be reasonably related to the study treatment or study participation, the Investigator must promptly notify the Sponsor or designee.

8.11.1 Definitions

Adverse Event

In accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E2A guideline Definitions and Standards for Expedited Reporting, an **Adverse Event (AE)** is any untoward medical occurrence or clinical investigation that is temporally associated with the use of a medicinal product or medical device which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease, temporally associated with the use of a pharmaceutical product, whether or not considered to be related to the pharmaceutical product or device.

A clinical laboratory abnormality will be reported as an AE/SAE if deemed to be clinically significant by the Investigator. Examples that may suggest clinical significance include:

- Accompanied by clinical symptoms.
- Leading to a change in treatment with IMP (e.g., dose modification, interruption, permanent discontinuation).
- The abnormality suggests a disease and/or organ toxicity.
- The abnormality is of a degree that requires active management (e.g., discontinuation of IMP, more frequent follow-up assessments, further diagnostic investigation).
- Any laboratory result abnormality fulfilling the criteria for an SAE will also be reported.

Serious Adverse Event

A **Serious Adverse Event (SAE)** is defined as any untoward medical occurrence that, at any dose:

- Results in death.
- Is life-threatening: the term "life-threatening" in the definition of "serious" refers to an event in which the participant was at immediate 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 to worsen or become more severe).
- Requires inpatient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability/incapacity.
- Results in a congenital abnormality, or birth defect.
- Is an important medical event: Medical events that may not result in death, be life-threatening, or require hospitalisation may be considered serious when, based on appropriate medical judgment, they may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in inpatient hospitalisation, or the development of drug dependency or drug abuse.

- Participants with DILI defined as AST or ALT g 3 × ULN; total bilirubin g 2 × ULN; absence of biliary obstruction/cholestasis (alkaline phosphatase < 2 × ULN) and no other explanation for the findings (e.g., viral hepatitis, chronic or acute liver disease, administration of concomitant hepatotoxic drug) must be reported as an SAE.

Adverse Reactions

An **Adverse Reaction (AR)** is a response to a medicinal product which is noxious and unintended, and which occurs at any dose. The term <reaction= means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

A **Serious Adverse Reaction (SAR)** is any adverse reaction (i.e., at least a reasonable possibility of a causal relationship with the medicinal product) that fulfils the criteria of seriousness, as defined above.

A **Suspected Unexpected Serious Adverse Reaction (SUSAR)** is a serious adverse reaction that is unexpected. The 8expectedness9 of a serious adverse reaction is assessed in the light of the reference safety information.

The **Reference Safety Information (RSI)** is a list of medical events that defines which reactions are expected for the IMP being administered to clinical trial participants, and do not require expedited reporting to the Competent Authority. It is contained within a specific section of the Investigator9s Brochure (IB) for non-marketed products and Summary of Product Characteristics (SmPC) for marketed products.

8.11.2 Assessment of Severity

Adverse events should be graded according to Table 9 below.

Changes in the severity of an AE/SAE should be documented to allow for assessment of the duration of the event at each level of severity. AEs/SAEs characterised as intermittent require documentation of the start and end of each incidence.

Adverse event severity and seriousness are assessed independently. 8Severity9 characterizes the intensity of an AE. 8Serious9 is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see above definition of SAE).

Table 9: Categorical grading of AE/SAEs

Grade	Global clinical assessment & application of grading to a diagnosis
Mild	Overall clinical condition is asymptomatic, or symptomatic with all the following characteristics: - No or minimal interference with usual social and/or functional activities. - No impact, or minimal impact (e.g., of short duration or a return to normal status without intervention), upon usual self-care activities. - Deviation from reference range for the population and from the participant9s clinically determined physiological baseline has no clinical impact and does not signal potential safety concern or unanticipated clinical risk. - The condition is reversible to baseline within an anticipated timeframe.

Grade	Global clinical assessment & application of grading to a diagnosis
	″ Intervention or treatment is either not indicated or indicated only to increase participant9s sense of wellbeing and comfort, but not due to safety concerns or risk.
Moderate	Overall clinical condition is asymptomatic or is symptomatic with no or minimal impact on usual self-care activities and one or more of the following characteristics: ″ Greater than minimal interference with usual social & functional activities. ″ Deviation from reference range for the population and from the participant9s baseline has clinical impact, but there is no acute safety concern or unanticipated risk. However, should the condition persist and/or occur more frequently, it could signal a potential safety concern or unanticipated risk and should therefore be monitored. ″ Reversibility of the condition to baseline condition takes longer than desirable or anticipated, impacting on the participant9s wellbeing beyond the mere severity of the condition. ″ Further medical assessment, intervention or treatment indicated to increase participant9s sense of wellbeing and comfort and to accelerate recovery and/or to prevent worsening.
Severe	Overall clinical condition is asymptomatic or is symptomatic with one or more of the following characteristics: ″ Significant impact on usual self-care activities. ″ Inability to perform usual social & functional activities. ″ The deviation from reference range for the population and from the participant9s baseline has significant clinical impact, it is an acute safety concern or unanticipated risk and requires close and continuous monitoring. ″ The reversibility of the condition to baseline condition takes significantly longer than desirable or anticipated, with significant impact on the participant9s wellbeing. ″ Intervention or treatment essential to treat the acute manifestations and to prevent worsening. ″ Life-threatening or Fatal

8.11.3 Assessment of Causality

The Investigator must assess, and document, the relationship of each AE/SAE to the IMP. Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to IMP administration must be assessed. The Investigator will also consult the Investigator9s Brochure (IB)/SmPC in their assessment.

The Investigator should also comment on the source document whether an AE/SAE is not related to the study treatment but is related to study participation (e.g., study procedures, wash-out periods etc.). AEs where a causal relationship between IMP and the AE is at least a reasonable possibility, are referred to as ARs.

Causality should be documented before the initial transmission of the SAE data to the Sponsor or Designee. The Investigator may change their opinion of causality with follow-up information and send an SAE follow-up report with the updated causality assessment.

The causal relationship of the AE/SAE will be assessed by the Investigator as either 8Related9 or 8Not Related9 as defined below.

Related: The available evidence suggests there is a reasonable possibility that the AE/SAE is caused by the IMP. The occurrence of the AE cannot be explained by other causative factors, but can be explained by the pharmacological effect of the IMP such as:

- Temporal relationship to IMP exposure.
- Event is known to be associated with the IMP drug class.
- Event improved on discontinuation or dose reduction of IMP.
- Event reoccurred on re-challenge of IMP.
- Biological plausibility.
- Other (must be specified).

Not Related: There is no reasonable possibility that the IMP caused the AE/SAE. The occurrence of the AE can be explained by other causative factors, such as:

- Event attributed to concomitant medication (provide details of the concomitant medication).
- No reasonably temporal relationship associated with IMP administration.
- Event is expected in the study indication and/or target population.
- Negative de-challenge and/or negative re-challenge.
- Other (must be specified).

8.11.4 Eliciting and Recording of Adverse Events

All (serious and non-serious) adverse events detected by the investigator or spontaneously notified by the participant at each visit/examination must be reported on the appropriate pages of the CRF. The following information should be reported for each adverse event, whether or not it can be attributed to study drug:

- Description of adverse event
- Date of onset/Stop date
- Characteristics of the event (seriousness, intensity)
- Actions taken (treatment required, or dose adjustments must be reported in the CRF)
- Outcome
- Relationship with study drug (causality assessment) and/or study participation

When an AE/SAE occurs, the Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information/documentation. Where feasible, the diagnosis will be documented as the AE/SAE. If a diagnosis is not available, each sign/symptom may be reported as a separate AE/SAE. If a diagnosis subsequently becomes available, this should be recorded, replacing the original entry(s) where appropriate. All relevant AE/SAE information must be recorded and filed in the CRF. SAEs will be recorded on the SAE form and filed in the CRF.

All AEs and SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate, or the investigator considers it medically justifiable to terminate the follow-up. The reason(s) will be recorded in the CRF when the AE follow-up is terminated.

8.11.5 Reporting of Serious Adverse Events

All AEs and SAEs must be reported regardless of the relationship to the study treatment. If an SAE occurs, the Investigators will take appropriate action immediately and will strive to identify the cause(s) of the events. Investigators should not delay the reporting of a suspected SAE to obtain

additional information. Any additional information, if collected, can be reported to the Sponsor or its designee as a follow-up to the initial report.

All SAEs/SUSARs will be notified by the Investigator to the Sponsor within 24 hours of awareness by email to:

E-mail: adverseeventreporting@orphalan.com

Phone: +356 99 966 819

Further follow-up SAE reports and any supplemental information will be provided as and when new information becomes available.

The Sponsor will also perform an evaluation of all SAEs prior to their finalization and/or reporting.

SUSARs will be notified to the MHRA and to the relevant REC(s) by Orphalan or its representatives within 7 days for fatal and life-threatening SUSARs or 15 days all other SUSARs.

Detailed reporting procedures will be outlined in the Pharmacovigilance Safety Management Plan.

8.12 Expedited Reports and Regulatory Requirements

8.12.1 Expectedness

The RSI for TETA 4HCL states that iron deficiency anaemia is an expected serious adverse reaction. However, given this is a healthy participant trial, there are no expected serious adverse reactions. All serious adverse reactions will be treated as unexpected and will be reported as SUSARs.

Prompt notification by the Investigator to the Sponsor or designee of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of patients and the safety of a study treatment under clinical investigation are met.

8.12.2 Regulatory Requirements

The Sponsor has a legal responsibility to notify the MHRA about the safety of a study treatment under clinical investigation. The Sponsor will comply with UK regulatory requirements relating to safety reporting to the MHRA, Institutional Review Boards (IRB)/IEC, and Investigators.

Suspected unexpected serious adverse reactions (SUSARs) will be notified to the MHRA by Sponsor or designee and to the relevant REC through combined review by Sponsor or designee within 7 (for fatal and life-threatening SUSARs) or 15 days (all other SUSARs), and forwarded to Investigators as necessary.

Annual safety reporting through combined review to the REC and MHRA will be in agreement with ICH guideline E2F <Note for guidance on development safety update reports (DSUR)=.

Investigators will be informed in writing of any new or emerging safety information as it becomes available. Notification of safety information to Investigators will be performed in accordance with the Pharmacovigilance Safety Management Plan.

8.13 Pregnancy

Pregnancy in itself, is not regarded as an AE, unless there is a suspicion that investigational product may have interfered with the effectiveness of a contraceptive medication. However, complications of pregnancy and abnormal outcomes of pregnancy are AEs and may meet criteria for an SAE. Complications of pregnancy and abnormal outcomes of pregnancy, such as ectopic pregnancy, spontaneous abortion, intrauterine foetal demise, neonatal death, or congenital anomaly, would meet the criteria of an SAE and therefore should be reported as an SAE as detailed above. Elective abortions without complications should not be handled as an AE.

If a female participant becomes pregnant during participation in the study, the participant must be withdrawn from study treatment, and the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If additional follow-up is required, the investigator will be requested to provide the information.

If a male participant9s female partner becomes pregnant while the participant was being treated or exposed to trial drug, consent must be obtained from the partner of the participant and the Investigator must report the pregnancy to Sponsor or designee using the Pregnancy Reporting Form. The participant partner should be followed until the outcome of the pregnancy is known. Male participants may continue in the trial if an accidental pregnancy of their female partner occurs despite adequate contraception. If additional follow-up of the female partner is required, the investigator will be requested to provide the information.

The pregnancy must be submitted to Sponsor or designee within 24 hours of awareness via the following:

E-mail: adverseeventreporting@orphalan.com

Phone: +356 99 966 819

9. QUALITY ASSURANCE AND QUALITY CONTROL

9.1 Quality Assurance and Quality Control

An inspection of this trial may be carried out by regulatory agencies. Such audits/inspections can occur at any time during or after completion of the trial. If an audit or inspection occurs, the PI and Richmond Pharmacology agree to allow the auditor/inspector direct access to all relevant documents and to allocate staff time to the auditor/inspector to discuss any findings or relevant issues.

Quality Control (QC) procedures at the Richmond Pharmacology will be implemented to ensure data recorded into the CRFs are accurate. QC checks will be carried out on an ongoing basis and according to the relevant SOPs. Records of QC checks will be documented and available for review.

Trial documents routinely undergo QC to ensure their accuracy before being use and/or sent to the sponsor. The Richmond Pharmacology staff member providing the QC checks the documents and notes any findings/comments on the QC record form. QC checks will be carried out on an ongoing basis throughout the trial and according to the relevant SOPs. Records of QC checks will be documented and available for review.

9.2 Monitoring

All aspects of the trial will be carefully monitored by the sponsor, or designee, for compliance with applicable government regulations with respect to Good Clinical Practice (GCP) and current standard operating procedures.

The monitoring of this trial will be performed by the sponsor9s monitor(s) or a designee in accordance with the principles of GCP as laid out in the International Conference on Harmonisation (ICH) Good Clinical Practice Guideline E6(R2) (2016).

The clinical monitor, as a representative of the sponsor, has an obligation to follow the trial closely. The monitor will maintain current knowledge of the trial through observation, review of trial records and source documentation, and discussion of the conduct of the trial with the investigator and staff. Further details will be described in the SOM.

10. STATISTICAL ANALYSIS

10.1 Statistical Analysis Plan

A statistical analysis plan (SAP) containing detailed statistical methodology will be written and signed off before the DB hard lock. The plan may be updated to reflect adaptive features of the trial as appropriate.

10.2 Analysis sets

The analysis of data will be based on different analysis sets according to the purpose of analysis. Participant eligibility for each analysis set will be finalised before the DB hard lock. A participant who withdraws prior to the last planned observation in a trial period will be included in the analyses up to the time of discontinuation.

Safety set
The Safety set will consist of all randomised participants who received at least one dose of the IMP. The safety set will be used for the safety analyses.

PK set
The PK set will consist of those participants in the safety set who have sufficient blood samples taken for at least one of the PK variables to be calculated. The PK set will be used for the PK analyses.

10.3 Statistical analysis of safety

Individual participant demographics (age, gender, and race) and body measurement data (height, weight, and BMI) at screening will be listed. These demographic characteristics and body measurements will be summarised by treatment group and overall, using the safety analysis set. Other baseline characteristics will be listed only.

AE data will be listed and summarised using descriptive statistics: the number (and %) of participants who had any AEs, and the number of AE episodes will be summarised for each dose. All AEs will be summarised and listed by using system organ class (SOC) and preferred term (PT) assigned to the event using Medical Dictionary for Regulatory Activities (MedDRA). Furthermore, these events will be summarised by the maximum intensity. The number of participants who had drug-related AEs will also be summarised. Any SAEs and/ or AEs that led to withdrawal will be summarised and listed.

Vital signs data (SBP, DBP, HR, Temperature, Respiratory rate) will be listed and summarised, along with changes from baseline, using descriptive statistics (mean, median, standard deviation, minimum, maximum). Out-of-reference-range values will be flagged as high (H) or low (L) and as being clinically relevant or not: the number of participants presenting out-of-range and clinically relevant values will be summarised.

All safety clinical laboratory data will be listed. Laboratory test results outside of the applicable range will be flagged as high (H) or low (L) and as being clinically relevant or not. The number of participants presenting out-of-range and clinically relevant values will be summarised. The quantitative laboratory data, along with changes from baseline will be summarised using descriptive statistics (mean, median, standard deviation, minimum, maximum). Change from baseline values at each assessment will be calculated as the assessment value minus the baseline value. The qualitative urinalysis data will be listed only.

All ECG data (PR, QRS, QT, QTcB, QTcF and HR) and overall ECG evaluation will be listed with comments. Out-of-reference-range values will be flagged. ECG data, along with changes from baseline will be summarised using descriptive statistics (mean, median, standard deviation, minimum, maximum).

Furthermore, categorical analysis of QTcF data will be presented as follows:

- Absolute QTcF interval prolongation
  - QTcF interval > 450 ms
  - QTcF interval > 480 ms
  - QTcF interval > 500 ms
- Change from baseline in QTcF interval
  - QTcF interval increases from baseline > 30 ms
  - QTcF interval increases from baseline > 60 ms

The number of participants presenting out-of-range and clinically relevant values will be summarised.

10.4 Pharmacokinetics

10.4.1 Evaluation of Pharmacokinetic Parameters

Non-compartmental analysis will be used for estimation of pharmacokinetic parameters.

The following pharmacokinetic parameters will be calculated for TETA 4HCL (active moiety, TETA)and its two main metabolites (N1-acetyltriethylenetetramine (MAT) and N1, N10-diacetyltriethylenetetramine (DAT)):

C_{max}	Maximal plasma concentration
C_{trough}	Plasma concentration prior to next dose
t_{max}	Time at which the maximum plasma concentration occurs
$t_{1/2}$	Terminal elimination half-life
AUC_{0-t}	Area under the plasma concentration curve from time zero up to the last quantifiable concentration
AUC_{0-24}	Area under the plasma concentration curve from time zero to 24 hours

$AUC_{0\text{-}inf}$	Area under the plasma concentration-time curve from time 0 extrapolated to infinite time
$\%AUC_{extrap}$	Percentage of AUC that is due to extrapolation from l_{ast} to infinity
CL/F	Apparent total plasma clearance
Vz/F	Apparent volume of distribution during the terminal phase
Rac	Accumulation ratio based on $AUC_{0\text{-}inf}$ and C_{max} after the first and the last dose

The individual plasma concentration data and the actual time for IMP administration and blood sampling will be used in the derivation of the PK parameters for TETA 4HCL (active moiety, TETA) and its two main metabolites (N1-MAT and N1, N10-DAT). If there is any doubt in the actual time a sample was taken, then the scheduled time will be used.

$AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ will be calculated using the linear/log trapezoidal method, applying the linear trapezoidal rule up to C_{max} and the log trapezoidal rule for the remainder of the curve. Samples below limit of quantification (LOQ) prior to the first quantifiable concentration will be set to zero. Samples with concentrations below LOQ after the first quantifiable concentration will be set to 8missing9 and omitted from the analysis. Other pharmacokinetic parameters will be calculated according to standard equations.

In the determination of $»_z$ (and $AUC_{0\text{-}inf}$), the following conditions should be met:

- A minimum of at least 3 data points in the terminal elimination phase, in which Cmax is not included;
- The Adj-Rsq should be g 0.8, and
- %AUCextrap < 20%.

If these conditions are not met, the PK parameter will be flagged in the listings (together with the ones dependent on $»_z$, such as $t_{1/2}$, CL/F and V_z/F) and they may be excluded from the summary statistics. The decision to include these parameters will be based on the decision of the Sponsor with input from the pharmacokineticist.

The following flags/footnotes may be applied to the pharmacokinetic parameters:

Flag	Footnote
a	Rsq of regression was <0.8
b	Period used for regression analysis was less than 2-fold the calculated half-life
c	Extrapolated portion of $AUC_{0\text{-}inf}$ >20%
d	Insufficient post-C_{max} data points for estimation of lambda-z
e	Entire profile BLQ, no pharmacokinetic parameters could be calculated
f	Regression line could not be fitted

Details will be provided in SAP.

10.4.2 Statistical Analysis on PK Parameters

Plasma concentrations will be listed and summarised by time point (N - the number of participants, n 3 the number of samples, n(LLOQ) - the number of samples <LLOQ, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum). Individual and arithmetical mean plasma concentration vs time curves for each analyte which includes all treatments will be produced on both linear/linear and log10/linear scales.

The PK parameters will be listed for each participant and summarized for each treatment group using descriptive statistics (N - the number of participants, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum).

For comparability of the two products the geometric mean, ratio of geometric means, confidence intervals (CI) including their logarithmic transformation together with the coefficient of variation for the within-participant variability will be summarised. In case a gender effect is noticeable, gender will be added as an additional term in the PK model, and all PK analyses will be presented per gender as well.

Details will be provided in SAP.

10.5 Handling of Missing and Incomplete Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) for handling missing data may be reassessed at the data review prior to database lock.

10.6 Determination of sample size

Formal sample size calculation for the comparison between the two treatments was performed based on a 2´2 crossover design. A sample size of 24 participants was selected to reach 91% power to infer that the mean difference is not 0. In comparison a sample size of 22 participants will reach 88% power based on two-sided t-test. This calculation was based on an actual mean difference of 1 and the square root of the within mean square error of 1 between test and reference formulations.

10.7 Interim Analysis

No interim analyses are planned.

11. DATA MANAGEMENT

Data Management will be performed by the Data Management department of Richmond Pharmacology. The data management process will be described in detail in the Data Handling Protocol (DHP).

The Richmond Pharmacology Data Management department will be responsible for developing and maintaining the DHP; setting-up and validating the clinical trial database; programming validation checks; entering data into the clinical trial database; reviewing data for accuracy, completeness, and consistency between the CRF and the database; and verifying adherence to the clinical pharmacology trial protocol and the DHP.

Clinical data queries will be generated and resolved according to the DHP. Clinical data queries are resolved with the assistance of Richmond Pharmacology clinical staff.

After all clinical data queries are resolved, final error rate is confirmed, and QC checks are acceptable the database will be locked.

Medical coding will be performed by Richmond DM. AEs, diagnoses from Medical History and procedures from Surgical History will be classified according to MedDRA. Concomitant medication will be coded using WHODRUG.

SAEs in the clinical database will be reconciled with the safety database.

Standard datasets are extracted from the final trial database and transferred to statistician for analysis.

11.1 Case Report Forms

A source data agreement will be signed by the sponsor and investigator to define what constitutes source data for all types of data captured.

Case Report Forms will be used to record data in the trial. Data should be recorded legibly onto the CRFs in black ballpoint pen. Correction fluid or covering labels must not be used.

The monitor will check data at the monitoring visits to the trial site. The PI will ensure by delegation that the data in the CRFs are accurate, complete, and legible.

Data from the completed CRFs will be entered into Richmond 9s clinical trial database and validated under the direction of the Data Manager. Screening failures (participants who signed consent to take part in the trial but were not randomised) as well as admission data for Reserves will not be entered into the clinical trial database. Any missing, impossible (inconsistent with human life), or inconsistent recordings in the CRFs will be queried to the PI and be documented for each individual participant before clean file status is declared.

12. REGULATORY, ETHICAL AND STUDY OVERSIGHT CONSIDERATIONS

12.1.1 Regulatory and Ethics Compliance

The study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the World Medical Assembly (Helsinki 1964) and subsequent amendments
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The procedures set out in this clinical study protocol are designed to ensure that Orphalan SA and any third party to whom aspects of the trial management or monitoring have been delegated abide by the principles of the current ICH GCP guideline on the conduct, evaluation, and documentation of this study, as described in ICH Topic E6 (Guideline for GCP). The study will also be carried out according to all applicable international and national regulatory requirements.

Visits to investigator sites will be conducted by representatives of Orphalan SA to inspect trial data, participants9 medical records, and CRFs in accordance with current ICH GCP E6 guidelines and the respective local and national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by competent authorities.

The investigator must undertake to perform the study in accordance with ICH GCP Guidelines, and the applicable MHRA regulatory requirements.

It is the investigator9s responsibility to ensure that adequate time and appropriate resources are available at the trial site prior to commitment to participate in this trial. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable participants within the agreed recruitment period.

The investigator will maintain a record of appropriately qualified persons to whom the investigator has delegated significant trial-related tasks. An up-to-date copy of the curriculum vitae for the investigator, sub-investigator(s), and essential trial staff will be provided to Orphalan SA (or designee) before starting the trial.

Agreement with the final Clinical Trial Report will be documented by the dated signature of the PI, in compliance with compliance with UK Statutory Instrument and ICH E3.

12.1.2 Medicines and Healthcare products Regulatory Agency (MHRA) and Research Ethics Committee (REC) Approval

Orphalan SA (or delegate) will ensure that MHRA requirements are met before the start of the trial.

It is the responsibility of the PI to submit this CSP, the informed consent document (approved by Orphalan SA), relevant supporting information, and all types of participant recruitment information to the REC and MHRA for combined review, and all must be approved prior to the start of participant screening. In addition, advertisements must be approved by the REC and MHRA prior to use at the site. Prior to implementing changes in the trial, Orphalan SA and the REC and MHRA must also approve any substantial amendments to the CSP and corresponding updates to informed consent documents. For non-substantial protocol amendments (that do not require regulatory approval) and subsequent updates of the ICF all changes will be done in agreement with Orphalan SA and Richmond Pharmacology.

12.1.3 Indemnity/liability and insurance

Orphalan SA will adhere to the recommendations of the Association of British Pharmaceutical Industry (ABPI) Guidelines. A copy of the Indemnity document will be supplied to the investigator before trial initiation.

Orphalan SA will ensure that suitable insurance cover is in place prior to the start of the trial. An insurance certificate will be supplied to Richmond Pharmacology.

12.1.4 Protocol management and adherence

All protocols and amendments will be prepared by Orphalan SA and/or Richmond Pharmacology. If it becomes necessary to issue a protocol amendment during the course of the trial, Orphalan SA will notify the investigator and collect documented Investigator Agreement to the amendment.

The PI and delegates must adhere to the CSP as detailed in this document. The PI will be responsible for including only those participants who have met CSP eligibility criteria. The PI will be required to sign an investigator Agreement to confirm acceptance and willingness for themselves and delegates to comply with the CSP.

12.1.5 End of trial notification and Clinical Trial Summary Results

Richmond Pharmacology on behalf of Orphalan SA will submit an end of trial notification to the MHRA within 90 days of the end of the trial in accordance with UK statutory requirements. The PI will be responsible for submitting these to the REC and MHRA through combined review within 90 days of the end of the trial.

For the purposes of this notification, the end of the trial will be defined as last participant, last visit.

Orphalan SA or its delegate will post result-related information from this clinical trial to Clinicaltrials.gov (which is considered as the submission of a summary of the clinical trial report) and send a confirmatory email to the MHRA, within one year of the end of the complete trial to the competent authority of the Member State concerned as required by the regulatory requirement and to comply with the Community guideline on Good Clinical Practice. Richmond Pharmacology on behalf of Orphalan SA will submit a summary of the clinical trial report to the concerned REC via combined review.

12.1.6 Documentation and retention of records

After completion of the trial, all essential records relating to the trial will be kept in an orderly manner and securely by the PI in a secure file and/or electronically, in compliance with ICH Good Clinical Practice Guideline E6(R2) (2016). The data will be available for inspection by Orphalan SA or their representatives. Essential documents must be retained for 25 years after the final marketing approval in an ICH region or at least 25 years have elapsed since the formal discontinuation of clinical development of Orphalan SA. The PI or delegate must contact Orphalan SA before destroying any trial-related documentation and it is the responsibility of Orphalan SA to inform the investigative site of when these documents can be destroyed. In addition, all participant records and other source documentation will be kept for a longer period if required by the applicable regulatory requirements.

12.1.7 Informed consent

The informed consent is a process by which a participant voluntarily confirms his/her willingness to participate in a clinical trial. It is the responsibility of the PI or delegate to obtain written informed consent from participants. All consent documentation must be in accordance with applicable regulations and the ICH Good Clinical Practice Guideline E6 (R2) (2016). Each participant is requested to sign the ICF after they have received and read the written participant information and received an explanation of what the trial involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the participant9s rights and responsibilities. Signed ICFs must remain on file and must be available for verification by Trial Monitors at any time. Another signed original of the ICF must be given to the participant or the participant9s legally authorized representative. The PI or delegate will submit ICFs to the REC via combined review and provide the sponsor with copies of the REC approved consent forms, and the REC written approval, prior to the start of the trial.

12.2 Confidentiality

For the purposes of this Section 12.4, <Applicable Data Protection Law= shall mean (a) the Data Protection Act 2018; (b), the UK GDPR (as defined in Section 3(10) of the Data Protection Act 2018) and (c) the General Data Protection Regulation ((EU) 2016/679) as applicable, and any applicable legislation introduced in the UK.

Data collected during this trial may be used to support the development, registration, or marketing of medicinal product. Orphalan SA will control all data collected during the trial and will abide by the Applicable Data Protection Law. For the purpose of the Applicable Data Protection Law, Orphalan SA will be the data controller. To the extent that Richmond Pharmacology processes personal data on behalf of Orphalan SA, in relation to such data Richmond Pharmacology shall only act in accordance with the terms of this protocol and Orphalan SA9s reasonable written instructions and Richmond Pharmacology shall take appropriate technical and organisational measures against the unauthorised or unlawful processing of such personal data.

After participants have consented to take part in the trial, their medical records and the data collected during the trial will be reviewed by Orphalan SA and/or its representatives. These records and data may, in addition, be reviewed by the following: independent auditors who validate the data on behalf of Orphalan SA; national or local regulatory authorities, and the REC which gave its approval for this trial to proceed.

Although participants will be known by a unique number, their date of birth will also be collected by Richmond Pharmacology and used to assist Orphalan SA to verify the accuracy of the data, for example, that the results of trial assessments are assigned to the correct participant. The results of this trial containing the unique number, date of birth, and relevant medical information including ethnicity may be recorded and transferred to and used in other countries throughout the world. If personal data is transferred outside the UK, Richmond Pharmacology will ensure applicable data transfer mechanisms are in place to ensure the data receives essentially equivalent protection as are applicable in the UK. The purpose of any such transfer would be to support regulatory submissions made by Orphalan SA in such countries. The Parties agree to comply with the relevant provisions of the Applicable Data Protection Law and any directions issued by the UK Information Commissioner9s Office in its processing of such Personal Data. All nominative information in the participant9s medical record will be kept strictly confidential. Nominative information shall mean the name, the address and all other personally identifiable information associated with a participant9s name. Orphalan SA access to participant9s data shall be performed in such a way that no participant could be identified by such data.

If there are any contradictions in terms of confidentiality requirements, the requirements of Applicable Data Protection Law will prevail.

To the extent that Richmond Pharmacology9s processes personal data on behalf of Orphalan SA, the terms are dictated in the MSA.

12.3 Publication Policy

Orphalan SA reserves the right to publish the trial's results independently, as they deem necessary for regulatory, scientific or commercial purposes. Richmond Pharmacology may also publish the trial's results, provided it is done in good faith and in collaboration with Orphalan SA. Richmond Pharmacology agrees to obtain Orphalan SA9s written approval for such publications or presenting trial results at scientific meetings and both parties are encouraged to work together to achieve timely and meaningful dissemination of findings.

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14. APPENDIX 1 3 CONTRACEPTIVE REQUIREMENTS

The contraceptive requirements for this trial have been created in coordination with the Clinical Trials Facilitation and Coordination Group (CTFG) <Recommendations related to contraception and pregnancy testing in clinical trials= guideline. This is based on possible human teratogenicity/fetotoxicity and no known genotoxicity[17]. The SmPC for Cuprior® has no specified contraceptive requirements.

Participants must agree to adhere to one of the following contraceptive methods to be eligible for trial enrolment (inclusion criteria 6):

a. Female participants of non-childbearing potential (WNCBP): Defined as either postmenopausal (evidence of menopause based on a combination of amenorrhea for at least one year and increased serum follicle-stimulating hormone (FSH) level [> 30 IU/L]), or surgical sterilization (evidence of hysterectomy and/or bilateral oophorectomy).

   CONTRACEPTION REQUIRED: None

b. Female participants of childbearing potential (WOCBP) who anticipate being sexually active with a male during the trial (from one complete menstrual cycle prior to the initial dosing until 1 month after the last dose in the study):

   CONTRACEPTION REQUIRED: Highly effective contraception must start one complete menstrual cycle prior to the first day of dosing and continue until 1 month after the last study dose. Highly effective contraception methods for WOCBP include:

   - Combined i.e. (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 hormone-releasing system (IUS)
   - Intrauterine device (IUD)
   - Bilateral tubal occlusion
   - Infertile male partner (e.g., vasectomised, permanently sterile following bilateral orchidectomy, or any other documented cause of infertility)

c. Female participants of childbearing potential (WOCBP) who agree to remain abstinent for the duration of the study (from one complete menstrual cycle prior to the first dosing until 1 month after the last dose in the study):

   CONTRACEPTION REQUIRED: Abstinence (N.B. sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the

entire period of risk associated with the trial treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the participant. Calendar, symptothermal and post-ovulation methods of contraception are not considered to be equivalent to abstinence).

d. Male participants, who agree to remain abstinent for the duration of the study (from first dosing until 1 month after the last dose in the study):

   CONTRACEPTION REQUIRED: Abstinence (N.B. sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the participant).

   If the situation changes post-dose during the trial, participants must use a condom.

e. Male participants, who anticipate being sexually active during the trial period (from initial dosing until 1 month after the last study drug administration) with a woman who is either a WOCBP, a woman who is pregnant and/or breast feeding:

   CONTRACEPTION REQUIRED: From the first day of dosing until 1 month after the last study drug administration . Acceptable methods are:

   - Male condom