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STATISTICAL ANALYSIS PLAN

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 (3x300mg Trientine Base Tablets, OD) with the Current Marketed Cuprior® Formulation (3x150mg Trientine Base Tablets, BD) in Adult Healthy Male and Female Participants

FINAL VERSION 1.0, 31JAN2024

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Contents

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
AE	Adverse Event
β-HCG	Beta-hCG, beta human chorionic gonadotropin
BD	Twice daily
BMI	Body Mass Index
CI	Confidence Interval
CV	Coefficient Of Variation
DAT	N1, N10-diacetyltriethylenetetramine
DRM	Data Review Meeting
ECG	Electrocardiogram
H	High
HR	Heart rate
IMP	Investigational Medicinal Product
L	Low
LLOQ	Lower Limit Of Quantification
MAT	N1-acetyltriethylenetetramine
MedDRA	Medical Dictionary for Regulatory Activities
OD	Once daily
PK	Pharmacokinetic(s)
Richmond	Richmond Pharmacology Ltd
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
TETA	Trientine
TEAE	Treatment-Emergent Adverse Event
WOCBP	Women Of Childbearing Potential

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the variables and provide details of the planned analysis for addressing the objectives of this trial.

The protocol dated 23 Nov 2023, version 1.1 was used to prepare this SAP.

Statistical analysis and reporting will be performed by Richmond Pharmacology (Richmond) as described within this SAP.

Trial OBJECTIVES AND ENDPOINTS

2.1 Trial objectives

Primary

- To evaluate and compare the plasma Pharmacokinetic (PK) parameters of TETA 4HCL (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.

Secondary

- To compare the safety and tolerability of the two TETA 4HCL tablet formulations.

2.2 Endpoints

Primary

- PK parameters derived by non-compartmental methods including maximum observed plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), 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-inf}), apparent total plasma clearance (CL/F), apparent volume of distribution during the terminal phase (V_z/F), terminal elimination rate constant (λ_z), and terminal elimination half-life ($t_{1/2}$).

Secondary

- 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).

3. TRIAL DESIGN

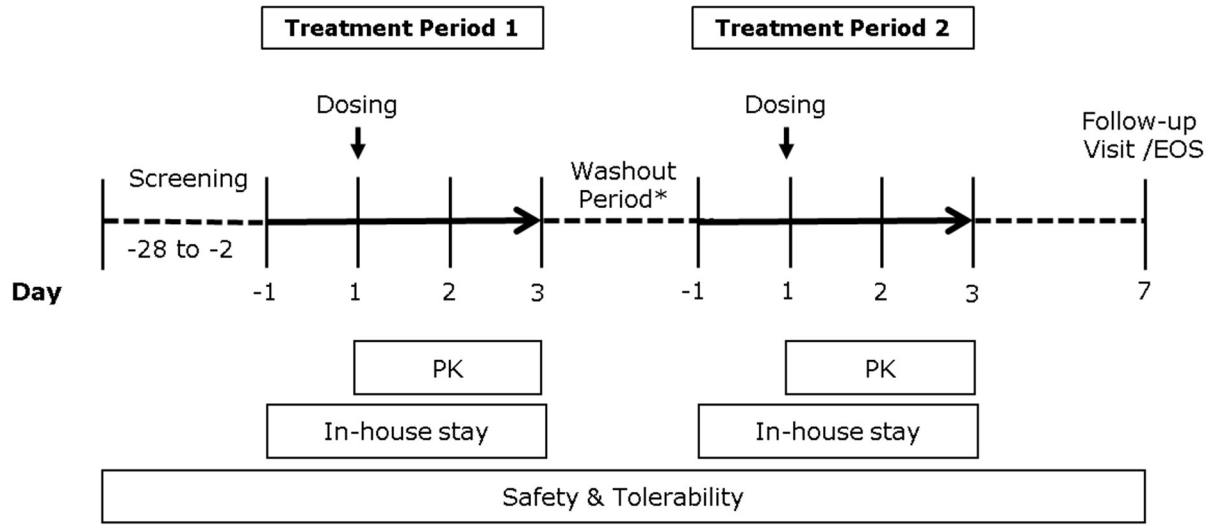
3.1 Overall Trial Design

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.

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 (β-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.

The overall trial design is depicted in Figure 1.

Figure 1: Study flow chart



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

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 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 1: 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 in protocol. The total duration of the study (including the screening period) is approximately 7 weeks.

3.2 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.

In the study 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.

4. STATISTICAL ANALYSES

4.1 General Notes for Statistical Analyses

Statistical analysis and reporting will be performed by Richmond.

In general, descriptive statistics for continuous variables will include number of non-missing values (n), arithmetic mean, standard deviation (SD), median, minimum, and maximum.

Categorical variables will be summarized using frequency counts and percentages.

For all tables, descriptive statistics for minimum and maximum will be presented with the same decimal digits as the original data, and with 1 more decimal place than the original data for mean and median; SD will be reported with 2 more decimal places than the original data.

To increase the readability of the TFLs the treatments will be abbreviated as

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

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

Listings and baseline data and Adverse event (AE) tables will be presented by treatment sequence AB, BA and Overall (where applicable). The analyses will be presented by treatment group (A and B), with pre-dose data presented under the treatment A.

All collected data will be presented in by-subject listings. Listings will be ordered by treatment sequence and subject number and will include all randomized subjects.

Unless otherwise stated, baseline will be defined as the last non-missing value prior to first administration of trial drug. Changes from baseline values will be calculated as the post-baseline assessment value minus the baseline value. Only observed values from scheduled time points will be used to create summary tables.

If repeated measurements are made at a time point, the first scheduled value will be used for summary analysis, unless otherwise stated in relevant section of this SAP.

Deviations from the planned analyses will be described in the final clinical study report.

4.2 Interim Analysis

No interim analysis is planned for this trial.

4.3 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 Investigational Medicinal Product (IMP) or Approved Product (150mg). 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.

4.4 Subject Disposition

All subjects will be included in the summary of subject disposition. This will present the overall number of subjects, the frequency and percentage of subjects randomized and treated, and who completed or discontinued from the trial, along with reason for discontinuation.

Furthermore, the number and percentage of subjects in each analysis set will be tabulated. Discontinued subjects will be listed. Subject assignment to analysis sets will be listed. Screen Failures will not be listed or included in summary tables.

4.5 Demographic Characteristics

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

4.6 Inclusion and Exclusion Criteria

The inclusion and exclusion criteria text will be listed, as well as failed eligibility criteria for each randomised subject, if any.

4.7 Protocol Deviations

The final review of protocol deviations will be performed at the DRM prior to database lock. The protocol deviations will be listed.

4.8 Medical and Surgical History

Medical and Surgical history data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary Version 26.1 (or higher) and listed individually. Surgical history data will be listed separately. Medical and Surgical history data will be summarised using frequency and percentage by SOC and preferred term.

4.9 Trial Drug Administration

Trial drug administration data including treatment details and date-time of administration will be listed by subject.

4.10 Prior and Concomitant Medications

All prior and concomitant medications will be coded using the World Health Organization Drug Dictionary version Sep 2023 (or higher) and will be listed individually. The frequency and percentage of prior and concomitant medications will be summarized by Anatomical Therapeutic Chemical and Preferred Name. Separate tables will be given for prior and concomitant medications. Prior medications are defined as those for which the end date and time is prior to the date, and time of first trial drug administration. Concomitant medications are defined as those with start date and time on or after the date and time of first trial drug administration and those with start date and time prior to the first trial drug administration but with end date and time on or after the date and time of first trial drug administration.

If medication dates are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant.

4.11 Safety Analysis

Safety analyses will be performed on the safety set, unless otherwise stated.

Safety analyses will include an analysis of all AEs, ECGs, clinical laboratory data, vital sign measurements and physical examination results and will be presented using descriptive statistics. No formal statistical analysis will be performed.

4.11.1 Adverse Events

A Treatment Emergent Adverse Event (TEAE) is defined as any adverse event which commences or any worsening of pre-existing conditions after the start of administration of trial drug. AEs with unknown start date/time will be assumed to be treatment-emergent unless the end date/time is known to be before the first administration of trial drug.

AE data will be listed and TEAEs summarised using descriptive statistics: the number (and percentage) of participants who experienced any AEs and the number of AE episodes will be summarized for each dose. All AEs will be summarized and listed by using the System Organ Class (SOC) and Preferred Term assigned to the event using the 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.

Subjects having multiple AEs within a category (e.g., overall, SOC and Preferred Term) will be counted once in that category. In each table, SOC and Preferred Term

will be presented in descending order of overall incidence rate (alphabetical order will be used in case of equal rates).

4.11.2 Laboratory Data

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.

The scheduled lab value will be used for summary analysis if repeated measurements are made at any time point.

For summary statistics, a lab value with "<" will be replaced with a numeric value by removing the "<" sign. In the listings, the values will be displayed as originally reported by the laboratory.

Serology, Urine cotinine, Pregnancy and Follicle-stimulating hormone tests will be listed only under a combined listing.

4.11.3 Vital Signs

Vital signs data (Systolic Blood Pressure, Diastolic Blood Pressure, Pulse rate, 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.

Normal ranges for the relevant parameters are presented below:

Parameter	Normal Range
Systolic Blood Pressure (Supine)	90–140 mmHg
Diastolic Blood Pressure (Supine)	40–90mmHg
Pulse Rate (supine)	40–100 bpm
Temperature (tympanic)	35-38°C
Respiratory Rate	8 – 20 breaths per min

4.11.4 Physical Examination

The full and symptom directed physical examination data will be listed only and include an assessment of the following: general appearance, skin, eyes, ears, nose,

neck, lymph nodes, throat, heart, lungs, abdomen, musculoskeletal system and extremities.

4.11.5 Electrocardiograms

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.

Reference ranges for ECGs are as follows:

Parameter	Normal Range
Heart Rate (Supine)	40-100 bpm
PR Interval	120–210 ms
QRS duration	≤120 ms
QTcF	≤450 ms (Males) ≤470 ms (Females)

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.

4.11.6 Other assessments

The meal times will be listed only.

4.13 Pharmacokinetic Data

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

The following pharmacokinetic parameters will be calculated for of trientine 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-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-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 of trientine 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-t} and AUC_{0-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 the lower limit of quantification (LLOQ) prior to the first quantifiable concentration will be set to zero. Samples with concentrations below LLOQ after the first quantifiable concentration will be set to 'missing' and omitted from the analysis. Other pharmacokinetic parameters will be calculated according to standard equations.

In the determination of λ_z (and AUC_{0-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 ≥ 0.8, and
- %AUCextrap < 0.20.

If these conditions are not met, the PK parameter will be flagged in the listings (together with the ones dependent on λ_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

4.13.1 Statistical Analysis on PK Parameters

Plasma concentrations will be listed and summarised by time point (N - the number of participants, n – 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.

4.14 Methods for Withdrawals, Missing Data and Outliers

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.