Statistical Analysis Plan

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1. List of Abbreviations and Definition of Terms

Abbreviation	Term	
AE	Adverse Event	
ALT	Alanine transaminase	
aPTT	Activated partial thromboplastin time	
AST	Aspartate transaminase	
BID	Twice a day	
BUN	Blood urea nitrogen	
CRF	Case report form	
GGT	Gamma glutamyl transpeptidase	
INR	International normalised ratio	
ITT	Intention-To-Treat	
LDH	Lactate dehydrogenase	
MedDRA	Medical Dictionary for Regulatory Activities	
NCC	Non-ceruloplasmin bound copper	
PP	Per protocol	
PT (AE)	Preferred Term	
PT	Prothrombin time	
RBC	Red blood cells	
SAE	Serious adverse event	
SOC	System Organ Class	
TETA 4HCl	Trientine tetrahydrochloride	
WBC	White blood cells	
WHO	World Health Organization	

2. Introduction

This Statistical Analysis Plan was written for the Phase 3 clinical trial GMPO-131-002 (CHELATE STUDY) conducted in 15 sites. The ICH guideline E3 "Structure and Content of Clinical Study Reports" was used as a guide to the writing of the plan.

3. Study Design and Objectives

3.1 Study Objectives

The primary objective of this study is to evaluate the efficacy of trientine tetrahydrochloride (TETA 4HCl) as compared to standard-of-care penicillamine, in adult patients with Wilson's disease who are adequately controlled and tolerating their penicillamine chelation therapy. The safety of TETA 4HCl compared to standard-of-care penicillamine will also be evaluated.

3.1.1 Efficacy endpoints

The primary endpoint of this study is the absolute value of non-ceruloplasmin bound copper (NCC) concentration (µg/L), measured at Screening/enrolment, Weeks 4, 8, 12, 16, 20, 24, 28, 32 and 36 (additional unscheduled measurements may also be taken). The primary hypothesis is that the efficacy of TETA 4HCl, as assessed by NCC levels, is not inferior to the efficacy of penicillamine, at Week 36.

The secondary efficacy measures are:

- 24-hour urinary copper excretion (µg/24 hours)
- Clinical global Impression of Change (CGIC) rating scale

Other efficacy measures are:

- Unified Wilson's Disease Rating Scale (UWDRS), neurological scale
- Serum total copper and serum ceruloplasmin

3.1.2 Safety endpoints

- Adverse events and serious adverse events
- Hematology, biochemistry and coagulation analysis
- Urinalysis
- Neurological signs and symptoms (using the relevant UWDRS items)
- Cognitive assessment using the semantic verbal fluency test
- Modified Nazer score
- Vital signs (heart rate, blood pressure, respiration rate, body temperature)
- Urine pregnancy test (for females of childbearing potential)

3.2 Study Design

This is a multicenter, randomized, open label study, comparing TETA 4HCl (tablets containing 150 mg of active product, individually dosed) with an active standard-of-care comparator, penicillamine (oral therapy, individually dosed). Patients will take their total daily dose as 2 divided doses (BID).

Patients who are considered to be stable on their penicillamine chelation therapy for at least 1 year will enroll in the study and enter a 12-week Penicillamine Baseline Period.

During this time all patients will continue to take their current penicillamine under study conditions.

At the end of the Penicillamine Baseline Period i.e. Week 12, patients who fulfill the protocol definition of being adequately controlled and tolerating penicillamine will be randomized in a 1:1 ratio to receive either TETA 4HCl (same total daily dose in mg as penicillamine dose at the end of the Penicillamine Baseline Period, rounded to the nearest 150 mg) or to continue to receive penicillamine (same total daily dose in mg as at the end of the Penicillamine Baseline Period). Post randomization there is a 24-week Post-randomization Phase. Scheduled study visits (clinic or telephone) will occur at Screening/enrolment (clinic), Week 4 (clinic), Week 8 (telephone), Week 12 (clinic), Week 16 (clinic), Week 20 (telephone), Week 24 (clinic), Week 28 (telephone), Week 32 (telephone) and Week 36 (clinic) – see Figure 1. For the telephone visits, the necessary lab samples will be collected directly from the patient.

On completion of 24-week Post-randomization phase at Week 36, patients will then have the opportunity to enter a 72-week extension phase where initially they will continue on their allocated treatment (penicillamine or TETA 4HCl) for a further 24 weeks i.e. until Week 60. The total daily dose will be the continuation of the patient's total daily dose in mg at the end of the 24-week Post-randomization phase at Week 36. Thereafter all patients will receive TETA 4HCl for a further 48 weeks and the total daily dose in mg of TETA 4HCl will be the same as the total daily dose the patient is receiving at the end of Week 60, rounded to the nearest 150 mg for those patients who were receiving penicillamine. Study clinic visits will occur every 24 weeks for all patients i.e. at Weeks 60, 84 and 108. An additional visit for patients who were previously allocated to the penicillamine treatment arm will occur at Week 64 (i.e. 4 weeks after starting to receive TETA 4HCl). Figure 1 depicts the overall study design. Table 1 shows the schedule of events.

Dose modification is permitted during the study.

Figure 1

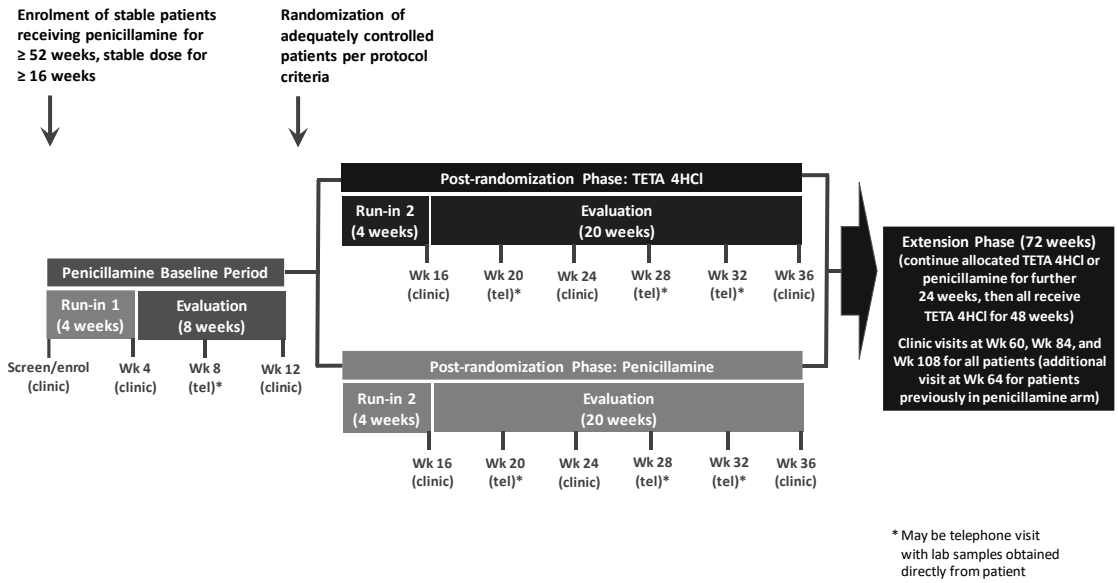

Table 1

Assessment	Day 1 Screening/ enrolment (clinic)	Week 4 ± 4 days (clinic)	Week 8[b] ± 4 days (tel)	Week 12 ± 4 days (clinic)	Week 16 ± 7 days (clinic)	Week 20[b] ± 7 days (tel)	Week 24 ± 7 days (clinic)	Week 28[b] ± 7 days (tel)	Week 32[b] ± 7 days (tel)	Week 36[i] ± 7 days (clinic)	Weeks 60, (64)[j], 84 ± 10 days (clinic)	Week 108[k] ± 10 days (clinic)
Signed informed consent and data protection[a]	X[r]											
Wilson's disease medical history and prior medication	X											
Other medical history and prior medication	X											
Demography, height/weight, HIV and viral hepatitis analysis	X[r]											
Vital signs	X	X		X	X		X			X	X	X
Non-ceruloplasmin bound copper[l]	X	X	X	X	X	X	X	X	X	X	X	X
Serum total copper[m]	X			X			X			X	X[q]	X
Serum ceruloplasmin[m]	X			X			X			X	X[q]	X
24-hour urinary copper excretion	X	X	(X – if repeat required)	X	X		X			X	X	X
Clinical Global Impression of Change		X		X	X		X			X	X	X
Hematology, biochemistry and coagulation analysis[c]	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis	X	X		X	X		X			X	X	X
Unified Wilson's Disease Rating Scale (Neurological scale)[n]	X[r]	X[r]		X	X		X			X	X	X
Cognitive assessment (semantic fluency test)[n]				X	X		X			X	X[q]	X

Assessment	Day 1 Screening/ enrolment (clinic)	Week 4 ± 4 days (clinic)	Week 8[b] ± 4 days (tel)	Week 12 ± 4 days (clinic)	Week 16 ± 7 days (clinic)	Week 20[b] ± 7 days (tel)	Week 24 ± 7 days (clinic)	Week 28[b] ± 7 days (tel)	Week 32[b] ± 7 days (tel)	Week 36[i] ± 7 days (clinic)	Weeks 60, (64)[j], 84 ± 10 days (clinic)	Week 108[k] ± 10 days (clinic)
Modified Nazer score	X	X		X	X		X			X	X	X
Recording of significant change in dietary copper intake		X		X	X		X			X	X	X
Samples for PK analysis[o]							X			X		
Urine pregnancy test[p]	X			X	X		X			X	X	X
Eligibility criteria	X	X[d]		X[d]								
Randomization				X								
Study medication dispensed	X[e]	X[e]		X[f]	X[f]		X[f]			X[g]	X[g]	
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events[h]	X	X	X	X	X	X	X	X	X	X	X	X

[a] Informed consent and data protection documentation must be signed prior to any study specific procedure being performed.
[b] For telephone visits, lab samples obtained directly from patient.
[c] Analysed by central laboratories.
[d] All criteria must still be fulfilled at Week 4 and at Week 12 (prior to randomization), including additional entry criterion regarding patient defined as adequately controlled and tolerating penicillamine.
[e] Study medication (standard-of-care penicillamine) taken from Day 1 (starting with the evening dose on the day of the screening/enrolment visit) to the Week 12 visit.
[f] Study medication (TETA 4HCl or continuation of standard-of-care penicillamine per randomized allocation) taken from Week 12 visit (starting with the evening dose on the day of the visit) to the Week 36 visit.
[g] For the Extension Phase, the allocated study medication (TETA 4HCl or standard-of-care penicillamine) will continue to be taken to Week 60 visit. Then all patients receive TETA 4HCl to Week 108 visit.
[h] Adverse events recorded from time of informed consent signature.
[i] End of 36-week phase of study/Exit if not continuing into extension phase (if a patient discontinues early during this phase, all effort should be made to perform all assessments).
[j] Week 64 visit only performed in patients previously allocated to penicillamine arm.
[k] End of the 18 month extension phase/Exit (if a patient discontinues early during this phase, all effort should be made to perform all assessments).
[l] Performed using validated assay (direct serum NCC assay) at a central laboratory .
[m] Performed using validated assays at a central laboratory, with results available at periodic intervals.
[n] Should be performed by an independent neurologist.
[o] Only for patients receiving TETA 4HCl at designated sites.
[p] For all women of childbearing potential. In addition, for any reported case of delayed menstrual period (over 1 month between menstruations) and for those women with

infrequent or irregular menstrual cycles, confirmation of absence of pregnancy should occur.
[q] Performed only at Week 60 visit.
[r] A patient may return to Day 1 if they do not fulfill the laboratory criteria defined by the inclusion/exclusion criteria. In this case these specified examinations do not need to be repeated.

3.3 Planned analyses

3.3.1 Interim Analysis

A pre-planned interim analysis will be performed when approximately 10 evaluable patients, who fulfil the protocol definition of being adequately controlled and tolerating penicillamine, have reached the Week 12 visit i.e. at the end of the Penicillamine Baseline Period, prior to randomization. Additional interim analyses may be conducted, if appropriate.

Only data collected at screening/enrolment and during the Penicillamine Baseline Period will be utilized for the interim analysis.

The purpose of the interim analysis is to perform an evaluation of the within-patient data for serum NCC in order to re-calculate the NI margin and sample size, if required (see Section 3.4). This will also be performed for the 24-hour urinary copper excretion.

3.3.2 Primary Analysis

The primary analysis of this study will occur following completion of the Week 36 visit, after all data are available. At this time, the database will be locked. Available data from the Extension Phase will also be evaluated at this time. However, they will be presented separately from the data analyzed in the primary analysis, which will only contain data up to Week 36.

The values of NCC used for the primary analysis will be those obtained using the selected, primary validated NCC assay. Analysis of NCC values using the initial assay for entry of the subject in the study will be supportive secondary analysis.

3.3.3 Additional Extension Phase Analysis

An analysis will be performed when all patients have completed the Extension Phase of the study.

Additional analyses after the primary analysis may occur as required e.g. to fulfill regulatory submission requirements.

3.4 Non-inferiority (NI) Margin and Sample Size Justification

There are no reliable historical data for within-patient serum NCC or 24-hour urinary copper excretion in stable patients with Wilson's disease on maintenance therapy, including comparison versus a control. The provisional NI margin and sample size calculation is based on data from 8 patients with stable Wilson's disease (Ala et al, 2015).

3.4.1 NI Margin

3.4.1.1 ***Provisional NI Margin***

In a series of 8 patients with stable Wilson's disease (Ala et al, 2015), the standard deviation of the within-patient measurements of serum NCC varied between 10 and 70 µg/L; so 50 µg/L was chosen as a plausible, provisional value for the non-inferiority margin of serum NCC.

Although not the primary endpoint, a NI margin is also provided for the 24-hour urinary copper excretion. The standard deviation of the within-patient measurements of urinary copper excretion in the aforementioned 8 patients varied between 1.4 and 276 µg/24 hours, which shows the large variability of urinary copper excretion; a 100 µg /24 hours

was chosen as a plausible, provisional value for the non-inferiority margin of serum urinary excretion.

3.4.1.2 ***Revised NI Margin***

At the time of the interim analysis (see Section 3.3.1) all available NCC data during the Penicillamine Baseline Period (using NCC values obtained from the initial NCC assay utilized for inclusion of the subjects in the study) will be utilized to assess the mean observed within-patient standard deviation (σ_{obs}).

In addition, the observed within-patient standard deviation for 24-hour urinary copper excretion during the Penicillamine Baseline Period will be evaluated.

In order to put these serum NCC data in context, per patient demography, baseline disease characteristics and prior/current treatment may also be provided.

If deemed appropriate, this may also be performed when more patients have completed the Penicillamine Baseline period.

In addition, for the primary analysis the final σ_{obs} for serum NCC during the Penicillamine Baseline Period will also inform the interpretation of the Non Inferiority (NI). This will be utilizing the values of NCC obtained using the selected, validated NCC assay for the primary analysis. The primary endpoints (i.e. the difference of NCC between the two arms at week 36) will be compared to the final within-patient standard deviation measured during the Penicillamine Baseline Period in order to provide hindsight on the Non Inferiority (NI) Margin. Similarly the difference of 24-hour urinary copper excretion between the two arms at week 36 will be compared to the final within-patient standard deviation measured during the Penicillamine Baseline Period in order to provide hindsight on the Non Inferiority (NI) Margin.

3.4.2 Sample size

3.4.2.1 ***Provisional Sample size***

The provisional sample is calculated under the following assumptions

- One-tailed significance level= 0.025
- Power = 0.8
- Observed standard deviation (SD) of within-patient difference in serum NCC= 60 µg/L
- NI margin for serum NCC (See Section 3.4.1.1) = 50 µg/L

With these assumptions, a sample size of approximately 46 patients is required. Therefore, approximately 55 patients will initially be planned to be randomized to account for drop-outs. This sample size is calculated under the conservative assumption that serum NCC will be compared at a single time point, whereas in fact the model will use repeated measures of serum NCC, thus providing more power for the comparison.

3.4.2.2 ***Sample Size Re-assessment***

The sample size will be recalculated at the time of the interim analysis (see Section 3.3.1), if needed, based exclusively on data from the Penicillamine Baseline Period, i.e. regardless of any difference between the randomized groups at the time of the pre-planned evaluation of the within-patient serum NCC and 24-hour urinary copper excretion data.

The interim analysis will be carried out by a statistician independent of the study team. A firewall will be put in place in such a way that the study team, i.e. the study statistician(s), statistical programmers and the people directly managing the sites, remains completely blinded to the details of the results of the interim analysis. In order to minimize any bias arising from the sample size re-assessment, participating investigators will also not be provided with details of the results of the interim analysis.

4. General Analysis Definitions

Data will be analyzed using SAS (Version 9.3 or higher). No tests of significance will be carried out to compare treatment arms on baseline data because any observed differences between them must be attributed to chance.

The significance level will be set at α = 0.05 for two-tailed tests, and at α = 0.025 for one-tailed tests.

Descriptive statistics will be tabulated as follows:

- Categorical data will be summarized in contingency tables presenting frequencies and percentages. Percentages will be calculated using the total number of patients in the population of interest in the denominator (See Section 4.1), or the number of patients with non-missing information. This will be specified in the tables.
- Continuous data will be summarized using number of non-missing values (n), mean, standard deviation, median, first and third quartiles, minimum and maximum values.

The tables will be created by treatment arm and overall, unless specified otherwise. Listings with individual values will be provided for all data presented in the tables.

4.1 Definition of Populations

4.1.1 Intention-To-Treat (ITT) Population

The Intention-To-Treat population (ITT) will consist of all randomized subjects, whether or not they receive one administration of study treatment post randomization. All subjects will be analyzed according to their randomized treatment allocation i.e. penicillamine or TETA 4HCl. This is the primary population for the primary efficacy analysis. All other efficacy analyses, except for those only including data of the Extension Phase, will also analyze the ITT population.

Data will be presented according to randomized treatment allocation i.e. penicillamine or TETA 4HCl and, in addition, for the Penicillamine Baseline period only, overall.

4.1.2 Per Protocol (PP) Population

The Per Protocol population (PP) consists of all patients without significant protocol deviations who complete up to Week 36 of the study. Subjects will be analyzed by actual treatment first received after randomization. The significant protocol deviations will be defined before database lock.

4.1.3 Safety Population

The Safety population will include all randomized subjects who received at least one dose of study medication post-randomization. Data will be presented according to the actual first treatment received after randomization i.e. penicillamine or TETA 4HCl and, in addition, for the Penicillamine Baseline period only, overall.

4.1.4 Extension Phase population

For the Extension Phase, efficacy and safety analyses will be based on patients who receive at least one dose of study medication in the Extension Phase. Subjects will be analyzed according to their actual treatment sequence received after randomization.

4.2 Treatment Assignment and Treatment Arms

Randomization will be performed through an interactive web based system. Patients will be centrally allocated in a 1:1 ratio to TETA 4HCl or penicillamine. A dynamic minimization procedure will be used, stratified by site and serum NCC concentration at Week 8 (≤ 75 µg/L or > 75 µg/L), using a stochastic treatment allocation algorithm based on the variance method (Freedman and White, 1976). This implementation of minimization guarantees that all treatment allocations are stochastic (Pocock and Simon, 1975).

4.3 Calculated Variables

- Baseline is defined as the last non-missing value of the Penicillamine Baseline Period, including unscheduled values.
- In case of repeated measure of lab values, the last value, i.e. the repeat result, will be used for analysis.

4.4 Partial Dates

Partial or missing dates in general will not be imputed, except for AEs' and laboratory information.

Partial adverse event (AE) start date and laboratory sample date will be imputed in order to include events and laboratory results in table summaries in case of doubt. The following rules will be applied:

- Partial date (only day missing):
  - If partial date falls in the same month as the first dose of randomized study treatment, then assign to first dose of study treatment + 1 day.
  - Else assign to the first day of the month.
- Partial date (day and month missing):
  - If partial date falls in the same year as the first dose of randomized study treatment, then assign to first dose of study treatment + 1 day.
  - Else if the partial date falls in the same year as a previous visit (before the first dose of randomized study treatment), then assign to this date of previous visit + 1 day.
  - Else assign to the first day of the year.

The imputed date will not be reported in the corresponding derived data listing. If a medication date is missing or partially missing, in a way that it cannot be determined if it was taken prior or concomitantly to the screening/enrolment visit (using the date and the eCRF variable that indicates if the medication was taken prior to the date of first screening/enrolment visit), it will be considered both as a prior and concomitant medication.

4.5 Methods To Be Used For Handling Missing Data

4.5.1 Missing data in the efficacy evaluation primary analyses.

If there are any missing data, the results obtained using a general linear model for correlated data, are valid under the missingness at random (MAR) assumption (See also Section 8.1.1). This means that results are valid, as long as the probability of missingness depends on the covariates in the model, and on observed outcomes, but not on the unobserved values of the endpoint. In this sense, imputation of missing data is not necessary, however a tipping point analysis will also be performed (see below).

4.5.1.1 Tipping point analysis

A tipping point analysis to evaluate sensitivity to potential violations of the missing data assumption (MAR) will be applied for the efficacy evaluation of serum NCC and 24-hour Urinary Copper Excretion at Week 36.

The general linear model described in section 8.1.1 will be used to estimate the:

- Differences between the means of the TETA and penicillamine arms at each timepoint: $\Delta = \mu_{p,x\ weeks} - \mu_{t,x\ weeks}$, …, with t=TETA and p=penicillamine
- The variance-covariance matrix of the repeated measures

All missing data will be imputed using SAS PROC MI procedure by using a Fully Conditional Specification (FCS) approach and the MNAR statement with the SHIFT option. Twenty different scenarios will be generated, in which the mean of the TETA arm will be gradually increased by steps of 10% of the estimated difference between the two arms. The marginal mean for imputing missing values according to scenario *k* will be

$\mu_{missing,\ t,\ timepoint\ i,\ scenario\ k} = \mu_{t,\ timepoint\ i} + \delta_{t,k}$. The space of plausible values for $\delta_{t,k}$ is defined as

$\delta_t \in [\Delta_{timepoint\ i} \times Coefficient_k]$,

With

- $[\Delta_{timepoint\ i}$ the difference between estimated TETA and pencillamine mean at timepoint *i*
- $\boldsymbol{Coefficient_k}$ going from 0.1 to 2 by steps of 0.1

For each of the 20 scenarios, 25 imputations of missing data will be performed. The missing values will be replaced by a value from a random number generator for the multivariate normal distribution using the assumption:

$missing\ values \sim N(\boldsymbol{\mu_{missing,\ t,\ scenario\ k}}, \boldsymbol{\Sigma})$

With

- $\boldsymbol{\mu_{missing,\ t,\ scenario\ k}}$ = the vector of shifted marginal means at each timepoint
- $\boldsymbol{\Sigma}$ the variance-covariance matrix estimated by the linear mixed model without imputation

The linear mixed models described in section 8.1.1 will then be applied on the 500 datasets (20 scenarios x the 25 imputations). The SAS procedure *proc mianalyze* will be used to combine the results of the 25 imputations linear mixed models of each scenario. A table, including the shift $Coefficient_k$, p-values and 95% confidence interval, will indicate if there is a tipping point that reverses the study conclusion.

4.5.2 Missing data in the non-primary analyses

Other missing, unused and spurious data will be treated as such and none of these data will be imputed, other than the aforementioned dates for AEs and laboratory information.

5. Study Patients

5.1 Disposition of Patients

The number of patients in each population, as defined in section 4.1, will be tabulated by randomized treatment arm and overall.

The number of patients treated and percentage of patients who prematurely discontinued post-randomization will be given by treatment arm and overall for the ITT population. The primary reason for premature discontinuation will be summarized. The details of the 'other reason' will be included in the listing.

Screen failures, including reason for failure will be tabulated separately.

5.2 In- and Exclusion Criteria

Listing of all in- and exclusion criteria not met will be provided by treatment arm. This will include all patients who entered the Penicillamine Baseline Period who did not get randomized (no treatment arm allocated).

6. Demographic and Other Baseline Characteristics

Descriptive statistics with respect to patient characteristics at baseline will be displayed for the ITT population by treatment arm and, where applicable for data collected in the Penicillamine Baseline Period (Period (up to and including Week 12), overall.

- Age, Gender and Race
- Height and weight
- Stratification factor: serum NCC concentration (≤ 75 µg/L or > 75 µg/L - value for the Week 8 visit)
- Wilson's disease medical history
    - Time since diagnosis (months)
    - Mutation analysis result (On both chromosomes detected/On 1 chromosome detected/No mutations detected)
    - Hepatic symptoms
        - At diagnosis
            - Clinical hepatic symptoms? (Yes/No)
            - Laboratory abnormality? (yes/No)
        - Approximately 1 year ago
    - Neurologic symptoms
        - At diagnosis (Yes/No)
        - Approximately 1 year ago (Yes/No)
    - Psychiatric symptoms
        - At diagnosis (Yes/No)
        - Approximately 1 year ago (Yes/No)
    - Other symptoms
        - At diagnosis (Yes/No)

- Approximately 1 year ago (Yes/No)

- HIV results
- Viral hepatitis results (Hepatitis C antibodies, hepatitis B surface antigen)
- Significant medical history (within the past 12 months prior to the screening/enrolment visit) other than Wilson's Disease will be tabulated by system organ class and preferred term for the ITT population, using the Medical Dictionary for Regulatory Activities (MedDRA Version 20.0).

7. Prior and Concomitant Treatment, prior and concomitant procedures and surgeries

Prior and concomitant medications will be classified according to WHO Drug Dictionary, starting with 2017 version 1 which will be upgraded each year. The most recent version in place at the time of database lock will be used (WHO Drug Dictionary 1 June 2020).

The number and percentage of participants receiving a prior or concomitant medication will be displayed by generic term and first Anatomical Therapeutic Chemical class (ATC 1) for each treatment arm and overall for the safety population.

ATC classes will be sorted by descending frequency. Prior and Concomitant medication summaries will be sorted alphabetically by generic term within ATC class. A listing of all medications recorded on the prior and concomitant medications CRF page will provide details including indication, dose, route, frequency, and start and stop dates.

- Will be considered as prior medications:
    - For Wilson disease: All medications taken prior to the screening/enrolment visit but stopped on or prior to the date of the screening/enrolment visit
    - For other disease: Medications taken in the 30 days prior to the screening/enrolment visit but stopped on or prior to the date of the screening/enrolment visit
- Will be considered as concomitant medications: medication continued to be taken after the screening/enrolment visit date, or initiated at or after the screening/enrolment visit.

  Prior and concomitant procedures and surgeries will also be tabulated by system organ class and dictionary-derived term.
- Will be considered as prior procedures and surgeries: prior to the screening/enrolment visit but stopped on or prior to the date of the screening/enrolment visit
- Will be considered as concomitant procedures and surgeries: continued after the screening/enrolment visit date, or initiated at or after the screening/enrolment visit.

  In addition, a per patient listing will be produced containing both the prior Wilson's disease medication as well as the study medication information, including details of dose and any dose modification during the study.

8. Efficacy Evaluation

8.1 Serum NCC Values

8.1.1 Difference at Week 36

The primary variable of absolute serum NCC values at Screening/enrolment, Weeks 4, 8, 12, 16, 20, 24, 28, 32 and 36, will be analyzed using a restricted maximum likelihood

(REML) based general linear model (GLM) for correlated data. The analyzed populations will be the ITT and PP populations (See Section 4.1).

The correlation due to repeated measures will be modelled by specifying the variance covariance matrix. In particular, the model will assume a general (unstructured) variance-covariance matrix for the measurements. If convergence problems are noted with the general variance-covariance matrix, a simpler structure of variance-covariance matrix will be considered: in hierarchical order, the Heterogenous Toeplitz (TOEPH), the Heterogeneous First-Order Autoregressive (ARH(1)) and the heterogeneous compound symmetry structure (CSH). If there are any missing data, the results obtained using GLM are valid under the missingness at random (MAR) assumption, i.e., as long as the probability of missingness depends on the covariates and observed outcomes, but not on the unobserved values of the endpoint. The Kenward-Roger approximation will be used to estimate the denominator degrees of freedom. The test for the treatment effect at the endpoint visit (Week 36) will be obtained by using an appropriate contrast of the model parameters. Non-inferiority of TETA 4HCl compared to penicillamine will be declared if the limit of the one-sided 97.5% confidence interval of the difference in mean serum NCC level under these two treatments excludes the non-inferiority margin.

An example of SAS code corresponding to the GLM described above is provided below:

```
proc mixed data=data method=reml;
class subject treatment visit site;
model serum_NCC=treatment visit site treatment*visit /ddfm=kr;
repeated visit/ subject=subject type=un;
estimate 'Penicillamine vs. TETA 4HCl  at Week 36' treatment 1 -1 treatment*visit 0 0 0 0 0 0
   0 0 0 1 0 0 0 0 0 0 0 0 0 -1 /CL;
run;
```

with:
subject = subject id
treatment = treatment group (penicillamine or TETA 4HCl)
visit = scheduled visit time

8.1.2 Difference at Week 60

Absolute serum NCC values at Screening/enrolment, Weeks 4, 8, 12, 16, 20, 24, 28, 32, 36 and 60 will be analyzed in the same way as in Section 8.1.1. The analyzed population will be the ITT population.

8.1.3 Summary Statistics

Summary statistics will be presented at all visits, both for absolute values and change from baseline, including observed (arithmetic) mean, least-squares mean plus 95%CI (provided by the models described in Section 8.1.1 and 8.1.2 above), median, standard deviation, interquartile range, minimum and maximum. For the Penicillamine Baseline Period (up to and including Week 12) data will be presented by treatment arm and overall.

8.1.4 Serum NCC Thresholds

By visit, the number and percent of subjects will be summarized for all visits and for the following serum NCC thresholds, including 95% confidence interval. The proportions of the two arms will be compared with a Fisher Exact test.

- Serum NCC ≤50 µg/L
- Serum NCC ≤100 µg/L
- Serum NCC ≤150 µg/L

- Serum NCC between ≥ 25 and ≤150 µg/L
- Serum NCC between >150 µg/L

The proportion of patients above and below each of these categories will be computed. Data will be presented according to randomized treatment arm, and for visits in the Penicillamine Baseline Period only, overall. If deemed appropriate new thresholds will be used.

These thresholds will also be used to summarize the serum NCC level by periods:

- The number and percent of subjects for whom the serum NCC measured during Penicillamine baseline visits are within the threshold values.
- The number and percent of subjects for whom the serum NCC measured from week 12 visit (excluded) until week 36 (included) are within the threshold values.
- The number and percent of subjects for whom the serum NCC measured from week 36 visit (excluded) until week 60 (included) are within the threshold values.
- The number and percent of subjects for whom the serum NCC measured from week 60 visit (excluded) until week 108 are within the threshold values.

8.1.5 Serum NCC within subject variability

The within subject variability of serum NCC will be estimated for each patient:

- during the Penicillamine Baseline Period and
- during the post randomisation phase (week 12 [excluded] to week 36 [included], week 36 [excluded] to week 60 [included]).

Descriptive statistics will present the within subject standard deviation by treatment arms (TETA/Penicillamine) and overall for the Penicillamine Baseline Period and for the post randomisation phase only by treatment arms.

A general linear mixed effects model will be applied to test the equality of the within patient post-randomization variability between the two treatments arms. An example of SAS code corresponding to the model mentioned above is provided below:

```
proc glimmix data=test;
      class timepoint arm usubjid;
      model Y=  arm timepoint/solution;
      random intercept / subject=usubjid;
      random timepoint / subject=usubjid type=un(1) residual group='arm';
      covtest homogeneity;
run;
```

8.1.6 Visualization of the evolution of Serum NCC

The Serum NCC will be plotted in order to show its evolution by subjects and by treatment arms.

8.2 24-hour Urinary Copper Excretion

8.2.1 Difference at Week 36

Values at Screening/enrolment, Weeks 4, 12, 16, 24, and 36 will be analyzed as in Section 8.1.1. The analyzed population will be the ITT population.

8.2.2 Difference at Week 60

Values at Screening/enrolment, Weeks 4, 12, 16, 24, 36 and 60 will be analysed in the same way as in Section 8.1.1. The analysed population will be the ITT population.

8.2.3 Summary Statistics

As described in Section 8.1.3

8.2.4 Urinary Copper Excretion Thresholds

By visit, the number and percent of subjects will be summarized for the following urinary copper excretion thresholds, including 95% confidence interval. The proportions of the two arms will be compared with a Fisher Exact test.

- 24-hour urinary copper excretion ≥ 100 µg/24 hours
- 24-hour urinary copper excretion ≥ 200 µg/24 hours
- 24-hour urinary copper excretion ≥ 300 µg/24 hours
- 24-hour urinary copper excretion ≥ 400 µg/24 hours
- 24-hour urinary copper excretion ≥ 500 µg/24 hours
- 24-hour urinary copper excretion ≥ 600 µg/24 hours
- 24-hour urinary copper excretion ≥ 700 µg/24 hours
- 24-hour urinary copper excretion ≥ 800 µg/24 hours
- 24-hour urinary copper excretion ≥ 900 µg/24 hours
- 24-hour urinary copper excretion between ≥ 100 and ≤ 900 µg/24 hours
- 24-hour urinary copper excretion between ≥ 100 and ≤ 400 µg/24 hours
- < 200 µg/24 hours

The proportion of patients above and below each of these categories will be computed. Data will be presented according to randomized treatment arm, and for visits in the Penicillamine Baseline Period only, overall. If deemed appropriate new thresholds will be used.

These thresholds will also be used to summarize the urinary copper excretion level by periods:

- The number and percent of subjects for whom the urinary copper excretion measured during Penicillamine baseline visits are within the threshold values.
- The number and percent of subjects for whom the urinary copper excretion measured from week 12 visit (excluded) until week 36 (included) are within the threshold values.
- The number and percent of subjects for whom the urinary copper excretion measured from week 36 visit (excluded) until week 60 (included) are within the threshold values.
- The number and percent of subjects for whom the urinary copper excretion measured from week 60 visit (excluded) until week 108 are within the threshold values.

8.2.5 24-hour urinary copper excretion within subject variability

The within subject variability of 24-hour urinary copper excretion will be estimated as described in section 8.1.5.

8.2.6 Visualisation of the evolution of 24-hour urinary copper excretion

The 24-hour urinary copper excretion will be plotted in order to show its evolution by subjects and by treatment arms.

8.3 Adjudication results

Each subject clinical stability is assessed by an independent clinical adjudication committee prior to Week 12 (randomisation) to confirm the subject is considered well-controlled and clinically stable prior to randomisation. The assessment of whether each subject is considered well-controlled and clinically stable is also performed post-randomization at Week 36 and at Week 60 by the independent clinical adjudication committee, who are blinded to the randomized treatment allocation.

The proportion of clinically stable subjects at Week 36 and Week 60 will be computed and reported with their exact 95% confidence interval. A Fisher Exact test will compare the two treatment arms.

8.4 Combined Outcomes

By visit, the number and percent of subjects will be summarized for the outcomes, including 95% confidence interval. The proportions of the two arms will be compared with a Fisher Exact test.

Combined outcome number 1

- serum NCC between ≥ 25 and ≤150 µg/L

AND

- 24-hour urinary copper between ≥ 100 and ≤ 900 µg/24 hours

Combined outcome number 2 (at Week 36 and Week 60 only)

- Combined outcome number 1

AND

- Adjudication results

Combined outcome number 3 (at Week 36 and Week 60 only)

- Combined outcome number 2

AND

- Adjudication results

If deemed appropriate, different or additional thresholds may also be selected.

8.5 CGIC Score

The secondary variable of CGIC score (7 points ordinal score), including all available values, will be summarized by visit.

At Week 36 and Week 60, the difference between the randomized groups will be tested using a stratified Cochran-Mantel-Haenszel test with modified ridit scores using the ITT population, based on the strata as defined in Section 4.2 (site and serum NCC concentration).

8.6 UWDRS Neurological Scale

The UWDRS scores (including all available values) will be summarized descriptively.

Summary statistics including change versus baseline will be presented by visit, for the overall neurological score, and for the two sub-components: patient assessment component and physician's assessment component. Data will be presented according to randomized treatment arm, and for visits in the Penicillamine Baseline Period only, overall

Also, all values will be graphically represented, depicting both individual and mean values, using spaghetti or comparable plots. This graphical representation may be done for the overall neurological score, the two sub-components, and all individual items.

The patient assessment component consists of the items 2 to 11: "mobility", "falling", "transfer", "salivation", "swallowing", "feeding", "dressing", "Taking a bath or a shower", "Grooming" and "Toilet use".

The physician's assessment component consists of items 1, and 12 to 34: "Consciousness", "Speech", "Facial expression", "Oculumotor function", "Tremor at rest", "Head tremor", "Rigidity", "Finger taps", "Rapid alternating movements of hands", "Handwriting", "Tremor in arms", "Finger-to-nose-test", "Leg ability", "Postural tremor in legs", "Cervical dystonia", "Arm and hand dystonia", "Arising from chair", "Posture", "Gait", "Chorea", "Jaw tremor", "Pyramidal signs", "Stereotyped actions" and "Involuntary crying"

8.7 Serum Total Copper and Serum Ceruloplasmin

Summary statistics will be presented by study visit, both for absolute values and change from baseline, including observed mean, standard error of the mean, 95% confidence interval for the observed mean, median, standard deviation, interquartile range, minimum and maximum.

Data will be presented according to randomized treatment arm, and for visits in the Penicillamine Baseline Period only, overall.

9. Safety Evaluation

9.1 General Definitions

Safety data will be tabulated separately for each of the 4 following study periods:

1. Screening/enrolment visit to Week 12 visit (included) i.e. Penicillamine Baseline Period. These tables will be created both overall and for the subset of randomized patients.
2. Week 12 visit (excluded) to Week 36 visit (included) i.e. the 24-week Post-randomization Phase
3. Extension Phase from Week 36 visit (excluded) up to Week 60 visit (included)

4. Extension Phase from Week 60 visit (excluded) to Week 108 visit (included)

Safety data will be tabulated by treatment sequence, based on actual received treatment, and overall:

- 'penicillamine/penicillamine'
- 'penicillamine/TETA 4HCl'
- 'Overall'

For the fourth period described above, i.e. the Extension Phase from Week 60 onwards, these columns will be named:

- 'penicillamine/penicillamine/TETA 4HCl'
- 'penicillamine/TETA 4HCl/ TETA 4HCl'
- 'Overall'

An AE is allocated to one of the 4 defined periods stated above according to the period in which the start date of the AE occurred. If an AE started in one period but was still ongoing in another period, it will only be allocated to the period in which the AE started.

AE listings will show the study period in which the AE occurred i.e. which of the 4 defined the AE occurred and number of days from the start of this period to the onset of the AE.

9.2 Extent of Exposure

A summary of the total daily dose (in mg) by treatment arm and overall will be done for the reported total daily dose at each visit, for the Penicillamine Baseline Period and post randomization, specifying if it is penicillamine or TETA 4HCl.

Also, for each visit, by period as defined in Section 9.1 and overall, the number of patients with dose modification will be summarized, along with the criteria for dose modification. Unscheduled visits with dose modification will be attributed to the first visit after the date of the unscheduled visit.

In addition, a per patient listing will be produced containing both the prior Wilson's disease medication as well as the study medication information, including details of dose and any dose modification during the study (see also Section 7).

Overall cumulative exposure to penicillamine and TETA 4HCl will also be summarized by providing the number of patients in the safety population (starting from screening/enrolment) who have received penicillamine or TETA 4HCl for ≥ 12 weeks, ≥ 24 weeks, ≥ 36 weeks and ≥ 52 weeks.

In addition to these summary statistics:

- The dose of each subject will be presented on graphs by treatments and arms.
- A listing of the dose adjustment and compliance will be presented by subjects.
- The visit window compliance will be presented in a listing

9.3 Adverse Events

All AEs occurring after enrolment into the study for the Penicillamine Baseline Period will be considered treatment-emergent.

Treatment emergent Adverse events (TEAEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA Version 20.0). TEAEs will be analyzed in terms of their type, incidence, severity and relationship to the study treatment.

Related TEAEs are defined as events with a relationship to study treatment equal to 'Possible', 'Probable', 'Definite' or with missing relationship.

Missing or partial AE start date will be estimated in order to include events in summary tables in case of doubt (see section 4.4 for more details).

A summary table will present by treatment arm the number and percentage of patients with at least one:

- TEAE
- Serious TEAE
- Moderate or severe TEAE
- TEAE leading to dose modification
- TEAE leading to dose increase
- TEAE leading to dose reduction
- TEAE leading to drug interruption
- TEAE leading to drug withdrawal
- Fatal TEAE

In addition, tabulations of the number of patients who experienced AEs as well as severity of the events will be presented by system organ class and preferred term. Patients will only be counted once for each preferred term. In case a patient experienced the same event more than once, the worst severity will be presented. In case of missing severity, it will be reported as missing. The following tabulations will be presented:

- All TEAEs
- TEAEs related to study treatment
- Severe TEAEs
- TEAEs leading to drug withdrawal
- Serious TEAEs
- Fatal TEAEs

Listings of all adverse events by treatment arm will be provided, including the patient identifier, age, race, sex, verbatim, preferred term, duration of the event, seriousness, fatality, other resulting medical event (life-threatening, hospitalization, cancer, congenital anomaly, other), severity, outcome, relationship to study drug, date of onset.

Separate listings will be produced for serious AEs, fatal AEs and severe AEs, similarly to the listing of all AEs.

9.4 Cognitive Assessment

The absolute values and change from baseline values will be summarized for each visit Data will be presented according to treatment arm, and for visits in the Penicillamine Baseline Period only, overall. Change from baseline will only be reported for post-randomization periods.

9.5 Clinical Laboratory Determination

The following categories of lab results will be presented: hematology (hemoglobin, hematocrit, RBC, platelet count, WBC, reticulocyte count), biochemistry (sodium,

potassium, bicarbonate, chloride, phosphorous, calcium, glucose, creatinine, uric acid, BUN or urea, total bilirubin, albumin, total protein, LDH, AST, ALT, GGT), coagulation analysis (PT, aPTT, INR), urinalysis (pH, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, specific gravity), pre-calculated Modified Nazer score as recorded in the eCRF, and urine pregnancy test.

Missing or partial lab dates will be estimated in order to include events in summary tables in case of doubt (see section 4.4 for more details).

Unscheduled visits will be imputed to the corresponding visit based on the lab date, applying rules of section 4.7 for missing/partial lab date. The unscheduled visit will be attributed to the closest scheduled visit in the same period, which could be before or after.

Lab data will be presented in two ways:

- Continuously:

Absolute values and change from baseline values will be summarized for each visit. Data will be presented according to treatment arm, and for visits in the Penicillamine Baseline Period only, overall. Change from baseline will only be reported for post-randomization periods.

- Categorically

The values will be compared to the limits of the normal range and classified as low, normal or high. In case of multiple values by visit, the value furthest from the upper/lower boundary of the normal range will be selected. For each parameter the worst treatment-emergent category will be tabulated by parameter and by period.

The pre-calculated CRF Modified Nazer score will only be summarized as a continuous variable.

Urine pregnancy test results (for those females of child bearing potential) will be summarized as a categorical binary variable.

Data will be presented according to treatment arm, and for visits in the Penicillamine Baseline Period only, overall.

9.6 Vital Signs

Descriptive statistics of systolic and diastolic blood pressure, heart rate, respiration rate and body temperature will be presented by visit. Both absolute values and change from baseline (only for post-randomization periods) will be presented. In case of multiple measurements by visit, the latest measurement will be used for baseline, the highest for post-baseline measures.

10. References

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