Multi-Center Study for the Assessment of Copper Parameters in Wilson Disease Subjects on Standard of Care Treatment

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Alexion Pharmaceuticals, Inc.

STATISTICAL ANALYSIS PLAN

PROTOCOL NUMBER: WTX101-203

MULTI-CENTER STUDY FOR THE ASSESSMENT OF COPPER PARAMETERS IN WILSON DISEASE SUBJECTS ON STANDARD OF CARE TREATMENT

Author: PPD

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1. APPROVAL SIGNATURES

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The abbreviations and acronyms in Table 1 are used in this Statistical Analysis Plan (SAP).

Table 1 Abbreviations and acronyms

Abbreviation or acronym	Explanation
AE	Adverse event
ALP	Alkaline Phosphate
ALT	Alanine aminotransferase (SGPT)
AST	Aspartate aminotransferase (SGOT)
ATC	Anatomical Therapeutic Chemical
BUN	Blood Urea Nitrogen
CGI	Clinical Global Impression
CGI-I	Clinical Global Impression – Improvement
CGI-S	Clinical Global Impression - Severity
CI	Confidence interval
cm	Centimeters
Cp	Ceruloplasmin
CPK	Creatinine Phosphokinase
CRF	Case report form
CS	Clinically significant
CTCAE	Common Terminology Criteria for Adverse Events
Cu	Copper
FA	Full Analysis
GGT	Gamma-glutamyl transferase
INR	International Normalized Ratio
kg	Kilogram
LSM	Least squares mean
MAR	Missing at random
MCID	Minimal clinically important difference
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
mg	Milligram
MMRM	Mixed model for repeated measures
Mo	Molybdenum
NCC	Non-ceruloplasmin-bound copper
NCI	National Cancer Institute
NCS	Not clinically significant
PP	Per-Protocol
PUF	Plasma ultrafiltrate
RBC	Red Blood Cell Count
SAE	Serious adverse event
SAS®	Statistical Analysis Software®
SAP	Statistical Analysis Plan
SD	Standard deviation
SEM	Standard error of measurement
SoC	Standard of Care
SOC	System Organ Class (MedDRA)
WCC	White cell count
WD	Wilson Disease
WHO	World Health Organization

4. DESCRIPTION OF THE PROTOCOL

Protocol Number:

WTX101-203 Amendment 1: 21SEP2016

Title of Program:

Multi-Center Study for the Assessment of Copper Parameters in Wilson Disease Subjects on Standard of Care Treatment.

Primary Objective:

To assess plasma and urine Copper (Cu) parameters in patients with Wilson Disease (WD) treated with Standard of Care (SoC) medications.

Secondary Objectives:

To compare Cu parameters with corresponding clinical data, including medical and medication history, clinical laboratory results, WD medications, and Clinical Global Impression (CGI).

Methodology:

This was a 24-month study to assess Cu parameters in patients with WD treated with SoC medications. Data were collected during routinely scheduled WD clinic visits at approximately 6-month intervals.

Data collected include: relevant medical history and WD medication history (including all Cu measurements and relevant clinical laboratory results for up to 5 years prior to study enrollment, if available), concomitant medications for up to 5 years prior to study enrollment, if available, blood samples for assessment of plasma Cu parameters and biochemistry, hematology and coagulation measures, urine from the routine 24 h-urine collection for assessment of Cu parameters, creatinine clearance, calculated Modified Nazer score, WD medications, and patients overall clinical status assessed by Clinical Global Impression (CGI) scale items 1 and 2. In addition to blood samples collected as SoC for analysis at the local laboratory, study blood samples of up to 30 mL were collected for analysis at the central laboratory at 8 time points (Enrollment, 1, 2, 3, 4, 12, 18, and 24 Months), i.e. at 5 routine clinic WD visits and at 3 additional time points.

After providing informed consent, patients meeting all inclusion and no exclusion criteria were enrolled into the study as outpatients. Subject's routine WD clinic visits were scheduled according to the standard clinical practice at the study center and at the discretion of the treating physician at approximate 6-month intervals. For the three blood sample collection time points at Month 1, 2 and 3 that did not coincide with a routine WD clinic visit, the sample was drawn, processed and shipped to the central laboratory either from the WD clinic, or from the subject's home (or subject-specified local address) by a qualified home health care nurse with experience in obtaining and processing blood specimens for clinical trials. Other than providing up to 30 mLs of blood for analysis at the central laboratory at each of the 8 blood collection time points, no other interventional assessment were required for subject participation in this protocol.

At the time of enrollment, patients were receiving SoC therapeutic agents for the treatment of WD. If treatment was interrupted or stopped during the course of the study, patients were to continue in the study and biological samples and clinical data were continuely collected for the full 24-month study period. Dosing with SoC agents, which can include penicillamine, trientine, zinc, or a combination of a Cu chelator and zinc, were individualized and managed by the treating physician at the study center according to standard clinical practice at the site.

Copper parameters, chemistry, hematology, 24-hour urine testing and other laboratory testing were performed by the site local laboratory as determined by the treating physician per the standard practice for the management of patients with WD.

In parallel with the local laboratory's analyses of routine biological samples, a central laboratory was utilized for the measurement of study-specific laboratory values, including ceruloplasmin (Cp), total Cu and Molybdenum (Mo), exchangeable Cu, speciation profiling, Cu and Mo in plasma ultrafitrate (PUF-Cu & Mo), urinary Cu and Mo, routine chemistry, hematology, coagulation testing and the required laboratory values were used to calculate the Modified Nazer score. The samples collected for the central laboratory testing were used to assess the Cu and WD related parameters, and the correlation of these parameters with corresponding clinical information.

Central laboratory results were transferred directly into a database by the central laboratory vendor. All other clinical data collected for the study (medical and medication history, 24h urine collection information, WD SoC medications, and CGI result) were entered into a web-based electronic data capture (EDC) system by the study physician or study coordinator/designee. The Modified Nazer score were calculated by a program in the EDC system for each of the visits.

Alexion Pharmaceuticals, Inc. or its designee will be responsible for writing the clinical study report.

Number of Subjects (Planned):

Approximately 60 patients will be studied under this protocol. Drop outs will not be replaced in this study.

4.1. Changes from Analyses Specified in the Protocol

Although it was planned to collect the plasma ceruloplasmin by enzymatic methodology but that test was not performed. Therefore, the analysis of ceruloplasmin by enzymatic test will not be included.

4.2. Changes from Analyses Specified in the Previous Version of the SAP

This is the first version of the SAP.

5. DEFINITIONS

5.1. Efficacy

5.1.1. Primary Endpoint

The primary efficacy endpoint is:

- The proportion of patients who achieve or maintain normalized levels of non-ceruloplasmin bound copper (NCC) (0.8-2.3 µM) or reach a reduction of at least 25% in NCC during 6 months of treatment if above the normal reference range at the time of enrolment

5.1.2. Secondary Endpoints

The secondary efficacy endpoints are:

- The proportion of patients who achieve or maintain normalized levels of NCC (0.8-2.3 µM) or reach a reduction of at least 25% in NCC after 6 months of treatment if above the normal reference range at the time of enrolment
- Change in 24-hour urinary copper
- Change in NCC levels
- Time to normalization of NCC if above normal reference range at the time of enrollment
- Change in exchangeable copper
- Change in copper ultrafiltrate
- Change in variables which could be used to calculate NCC (Copper Plasma, Ceruloplasmin Serum (nepholometry))
- Change in molybdenum (Molybdenum Plasma, Molybdenum Ultrafiltrate, Molybdenum Urine)
- Changes in hepatic measures (alanine aminotransferase (ALT), aspartate aminotransferase (AST), International Normalized Ratio (INR), and bilirubin)
- Changes in the Clinical Global Impression (CGI) scale items 1 (severity of illness) and 2 (global improvement)

To achieve a normalized NCC level, patients must have demonstrated 2 consecutive measures within (or below) the normal range (0.8-2.3 µM).

5.1.3. Other Efficacy Endpoints

The exploratory efficacy endpoints include the following:

- Change from baseline where average change will be presented with a 95% confidence interval (CI) for Model for End-Stage Liver Disease (MELD), and Modified Nazer;
- The proportion of patients whose score improved, deteriorated, or remained stable compared to baseline according to MELD and Modified Nazer;
- The proportion of patients who meet or exceed the minimal clinically important difference (MCID) for MELD and Modified Nazer;
- Correlations between results from NCC (described in Section 7.2) and the NCC direct assay;
- If there are sufficient samples for analysis, the primary and secondary efficacy endpoints utilizing NCC may be repeated using results from the NCC direct assay.

5.2. Safety

This is not an investigational drug study. With the exception of providing 8 blood samples and beyond those required for patients SoC therapy, patients will not undergo any other study-related interventional testing or assessments. Only adverse events (AEs) associated with study related interventional testing or assessments will be collected. Most will be venipuncture AEs. AE data will be collected regarding onset, duration, intensity, seriousness, and outcome.

The following AEs will not be collected during this study:

- AEs that may be associated with the specimen collection procedure but result only in local, mild and transient discomforts, such as hematoma, redness, slight discomfort.
- AEs related to the subject WD or treatment, or any other medical condition or events the subject experiences during the study.

Laboratory values will also be evaluated and assessed.

5.2.1. Adverse Events (AEs)

Patients will be provided with the investigator's contact information and will be instructed to notify the investigator of any AEs they experience during or secondary to the specimen collection procedures.

For the purposes of this study, an AE is defined as any undesirable physical, psychological or behavioral effect experienced by a subject in conjunction with the blood sample collection procedures described in this protocol for a period of up to 30 minutes post sample collection.

Each AE is to be characterized (i.e., verbatim term) and information provided regarding its start and stop dates, intensity, seriousness, and outcome. The safety evaluation will include an assessment of all AEs, SAEs, and AE intensity. Adverse event intensity will be evaluated using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE).

5.2.2. Laboratory Assessments

Laboratory data, including all available Cu information, and available relevant clinical laboratory measurements, will be collected retrospectively for up to five years prior to study entry if available.

Biochemistry, hematology, coagulation, and copper analyses testing will be performed at the visits of enrollment, 1, 2, 3, 4, 12, 18, and 24 Months. The specific laboratory assessments and further details are provided in Section 8.1 of the Protocol.

The following clinical laboratory measurements are considered relevant for assessing safety in this study:

Biochemistry: Creatinine (including calculated creatinine clearance by Cockcroft-Gault method), blood urea nitrogen (BUN), albumin, protein, total bilirubin, conjugated bilirubin, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), creatinine phosphokinase (CPK).

Hematology: Red blood cell count (RBC) (including nucleated RBC), white cell count (WCC), platelets, hemoglobin.

Coagulation: International Normalized Ratio (INR).

6. DATA SETS ANALYZED (STUDY POPULATIONS)

6.1. Full Analysis (FA) Set

Patients who have been enrolled in the study will be included in the full analysis (FA) set. (Note: To be eligibly enrolled, patients must have been receiving the standard of care therapeutic agents (penicillamine, trientine, zinc, or copper chelators with zinc) for the treatment of WD at the time of enrollment and for no more than 60 months prior to enrollment.)

6.2. Per Protocol (PP) Set

Patients meeting the definition of Full Analysis Set (Section 6.1) and who are without major protocol deviations that would impact the primary endpoints will be included in the per protocol (PP) analysis set. Further details are provided in Section 7.1.2.

6.3. Safety Set

The definition of Safety Set is the same as the FA set. The Safety Set will be used for all safety analyses.

7. STATISTICAL ANALYSIS

For the statistical analyses below, descriptive statistics (n, mean, median, SD, 95% CI, minimum, and maximum) will be provided for each continuous variable, and frequencies and percentages will be provided for each categorical variable. Certain categorical variables will be summarized as proportions with 95% CIs. All data will be displayed unless otherwise indicated. No formal hypothesis testing will be performed to compare differences between treatment cohorts. Analyses will be conducted using Statistical Analysis Software (SAS©) version 9.4 or higher.

In general, analyses will be presented by treatment cohort and overall where specified. Patients with prior WD treatment >28 days (treatment experienced) will be in Cohort 1 and patients with prior WD treatment ≤28 days will be in Cohort 2.

7.1. Study Patients

7.1.1. Disposition of Patients

An overview of patient populations will be summarized by treatment cohort. Frequency counts and percentages of patients excluded prior to enrollment will be provided for patients who failed to meet study entry requirements during screening. Additionally, a summary of patients who did not meet inclusion or met exclusion criteria will be provided.

The number and percent of patients completing the study will be described. For patients who discontinued the study, the reason for discontinuation, including death, will be summarized. Information will be reported using all enrolled patients and summary statistics will be presented by treatment cohort and overall. Patient disposition will also be summarized separately for each study center. A summary will be provided of patients by region, country and site.

A listing of patients will be provided for all enrolled patients. A listing of analysis populations for all enrolled patients and a listing of screen failure patients will also be provided. Additionally, a listing of the inclusion/exclusion criteria and a listing of patients and the inclusion criteria they failed to meet and the exclusion criteria they met will be provided.

7.1.2. Protocol Deviations

All protocol deviations will be classified into category of deviation and assigned major or minor status prior to database lock. These will be summarized with counts and percentages by both category and severity of deviation. Additionally, these will be presented in a listing including the category and major/minor status. A listing of the reasons patients were excluded from efficacy analyses, including the per protocol set, will also be provided.

7.1.3. Demographics, Disease Characteristics, and History

All demographic and baseline characteristics information are summarized using the FA Set. Summary statistics will be presented by treatment cohort and overall. A listing will also be provided.

7.1.3.1. Demographics

The following demographic variables will be summarized:

- Sex
- Ethnicity
- Age (years) at informed consent

7.1.3.2. Disease Characteristics

The following baseline disease characteristics will be summarized:

- Time since WD treatment start date (months)
- Molybdenum plasma (ng/mL)
- Copper Plasma (ng/mL)
- Ceruloplasmin Serum (nepholometry) (mg/L)
- NCC (µM)
- Exchangeable Copper (ng/mL)
- Molybdenum Ultrafiltrate (ng/mL)
- Copper Ultrafiltrate (ng/mL)
- 24 Hour Urinary Molybdenum Concentration(ng/mL)
- 24 Hour Urinary Copper Concentration(ng/mL)
- Clinical Global Impression Severity Scale (CGI-S)
- Albumin (g/L)
- Protein (g/L)
- Total Bilirubin (mg/dL)
- Direct (aka: Coagulated) Bilirubin (umol/L)
- Gamma-Glutamyl transferase (GGT) (U/L)
- Alkaline Phosphatase (ALP) (U/L)
- Alanine Aminotransferase (ALT) (U/L)

- Aspartate Aminotransferase (AST) (U/L)
- Creatinine Phosphokinase (CPK) (U/L)
- Creatinine (umol/L)
- Creatinine Clearance (Cockcroft-Gault) (mL/sec)
- Red Blood Cell Count (RBC) (10^6/µL)
- White Blood Cell Count (WCC) (10^3/µL)
- Platelets (GI/L)
- International Normalized Ratio (INR)

7.1.3.3. Medical / Surgical History

Medical and surgical history will be summarized by frequencies and percentages and displayed by System Organ Class and Preferred Term within each System Organ Class. System Organ Class and Preferred Term are coded using Medical Dictionary for Regulatory Activities (MedDRA), version 18.0. Medical and surgical history will also be presented in a listing including the medical history verbatim text describing each history event or first known symptom and any abnormal physical examination free text findings.

7.1.4. Prior and Concomitant Medications / Therapies

Medications are coded using the WHO drug dictionary and will be summarized by Anatomic Therapeutic Chemical (ATC) level 3 class and generic drug name.

Prior medication for WD was retrospectively collected up to five years. Prior and concomitant medications will be summarized for the FA set by treatment cohort and all patients combined. The number and percentage of patients receiving any concomitant medication will be summarized, as will the number and percentage receiving any concomitant medication by ATC drug class and generic drug name. Patients reporting use of more than one medication at each level of summarization (any medication received, ATC class, and generic drug name) will be counted only once. ATC class terms will be displayed by descending order of incidence, as will generic drug names within each ATC class. Prior medications used to treat WD and an additional analysis of all prior medications will be summarized similarly.

Prior medications will be defined as medications that discontinue prior to the baseline visit. Concomitant medications will be defined as medications that either started prior to baseline visit and were continuing at the baseline visit, or started on or after the baseline visit. If it cannot be determined whether a medication was stopped prior to the baseline visit due to partial or missing medication start or end dates, it will be considered a concomitant medication. Further details on partial missing dates can be found in Appendix 9.4.

Prior and concomitant medications will be presented in a listing by patient and medication name.

7.2. Efficacy Analyses

The analyses and summaries will be based on the FA Set. Additionally, the primary analyses will be performed on the PP Set. For by-visit analyses, the definition is given in Appendix 9.4. Listings will also be provided for all efficacy assessments. Graphical displays will be provided for the Cu parameters over time.

7.2.1. Primary Analysis

NCC is calculated by subtracting the amount of Cu bound to ceruloplasmin from the total plasma Cu level:

$$NCC[\mu M] = \frac{Total\ plasma\ Cu\ [\mu g/L] - (3.15 * ceruloplasmin\ [mg/L])}{63.5\ [\mu g/\mu mol]}$$

In the calculation of NCC, the following adjustments will be made:

- Copper values <LLOQ are set to missing and NCC will not be derived;
- Ceruloplasmin <LLOQ are set to 0;
- Negative NCC values are set to missing;

The independent variable (X) represents NCC, with the proportional change from baseline as follows:

$$Y_t = \frac{X_t - X_0}{X_0}$$

Where X_0 is the copper level at baseline and X_t is the copper level at time t.

The criteria for success is defined as follows:

- Success = Two consecutive measurements with Y < -0.25, i.e., a reduction of the copper level of at least 25%) or two consecutive measurements of normalized (or lower) levels of NCC (0.8-2.3 µM) will be considered a success. Also, for patients with normal copper levels (or lower) at baseline, maintenance of normal levels (or lower) is considered a success.
- Non-success = Patients who do not reach the success-criteria within 6 months.

Two consecutive measurements requires that the measurements occur on separate dates and are assigned to two different visits under the visit windowing rules given in Appendix 9.4. However, if a subject is missing a value at a scheduled visit, it is possible that the two consecutive measurements do not occur on consecutive visits per the schedule of events (e.g. patient could be missing a value at Month 3 and so the measurements from Month 2 and Month 6 would be

considered consecutive). The primary efficacy endpoint is the proportion of successful patients. The primary endpoint will be analyzed using descriptive statistics but also by means of confidence interval based on the exact binomial method for the proportion of successful patients.

Summary of success will be summarized for the FA Set and for the PP Set.

7.2.1.1. Handling of Dropouts or Missing Data

In the analysis of the proportion of successful patients for the primary efficacy endpoint, patients with missing data at each time point will be considered non-successful. In the analysis of continuous secondary efficacy endpoints, mixed model for repeated measures (MMRM) analyses will be performed to mitigate the impact of missing data. This approach assumes that missing observations are missing-at-random (missingness is related to observed data) during the study and borrows information from other patients in the same treatment cohort taking into account both the missingness of data and the correlation of the repeated measurements.

7.2.1.2. Subgroup Analysis

Efficacy endpoints may be summarized by various subgroups of interest, including the following:

- The primary efficacy endpoint (Section 5.1.1) and secondary efficacy endpoints (Section 5.1.2) will be repeated by baseline NCC level ($\leq$2.3 µM vs >2.3 µM);
- MELD, for the patients with elevated MELD at baseline, defined as MELD >6;
- Modified Nazer, for the patients with elevated Modified Nazer score at baseline, defined as Modified Nazer >0

Demographic and baseline disease characteristics will also be produced for the aforementioned subgroups. Additional subgroup analyses may be performed post-hoc, as appropriate.

7.2.1.3. Multicenter Studies

This is a multicenter study, with up to 15 centers in North America and Europe expected to participate. Efficacy data collected from all study centers will be pooled for data analysis.

7.2.1.4. Hypothesis Testing and Significance Level

No formal hypothesis testing will be performed to compare differences between treatment cohorts.

Within treatment cohorts, change from baseline may be tested using a two-sided test at the alpha=0.05 level of significance. If the p-value is less than 0.05, a significant change from baseline will be claimed. All hypothesis testing and other inferential statistics should be considered purely nominal, as the study was not powered for them and no adjustments will be made for multiple comparisons.

7.2.1.5. Sensitivity Analyses

There are no planned sensitivity analyses.

7.2.2. Secondary Analyses

The secondary endpoint analyses will be conducted on the FA Set. Efficacy endpoints will be summarized by treatment cohort, as described in Section 7. Multiple records may exist for some efficacy endpoints (e.g. total plasma copper and total plasma molybdenum) with the same date and time and when this occurs, the records will be averaged. A summary for "Last Assessment" will be included for the last available post-baseline result for each patient.

Similar methods as for the primary endpoint will be employed in the FA set for the proportion of patients who achieve or maintain normalized levels of NCC (0.8-2.3 μM) or reach a reduction of at least 25% in NCC after 6 months of treatment if above the normal reference range at the time of enrollment.

Change in 24-hour urinary copper will be analyzed by a hypothesis test to evaluate if there is a significant average reduction compared to baseline. Change in NCC levels at all post-baseline visits will be analyzed with a similar method. Graphical displays will be provided.

For the time-to-normalization variable, a Kaplan-Meier approach will be used where patients not normalized will be censored at the latest observed time point. The group reaching normalization will be summarized by descriptive statistics and plotted. This analysis will only be performed on the subset of patients with elevated copper levels (>2.3 μM) at baseline. To achieve a normalized NCC level, patients must have demonstrated two consecutive measures within the normal range ($\leq$ 2.3 μM).

Most secondary endpoints are calculated as change from baseline and percent change from baseline. For these variables (exchangeable copper, copper ultrafiltrate, variables which could be used to calculate NCC, molybdenum, and hepatic measures), the average change will be presented with a 95% CI. Even though the main focus is descriptive, the CIs will give an indication of whether a significant change from baseline has been achieved or not. The secondary endpoints will also display percent change from baseline. For endpoints which do not require change from baseline (e.g. CGI-Improvement scale), the results at each time point will be summarized. Graphical displays will be provided

Additional analyses for the secondary continuous efficacy endpoints will be conducted using a mixed model repeated measures (MMRM) on the FA Set. The response variables will be the change from baseline and percent change from baseline of NCC and CGI Severity Scale. The model will include factors for baseline, treatment cohort, visit (all post-baseline visits per response variable), and visit-by-treatment cohort interaction. The Restricted Maximum Likelihood estimation will be used. An unstructured covariance matrix will be used to model the within-subject error and the Kenward-Roger approximation will be used to estimate the degrees of freedom. If the fit of the unstructured covariance structure fails to converge or a positive definite Hessian matrix is not produced, the following covariance structures will be tried in order

until convergence is reached: toeplitz with heterogeneity, autogressive with heterogeneity, toeplitz, autoregressive, and compound symmetry. Based on the model, the comparison of post-baseline vs. baseline time points will be tested using a two-sided test at the alpha=0.05 level of significance. The least squares mean (LSM) change from baseline and associated SE will also be presented along with the 95% CI. If the number of observations at a visit within a treatment cohort are fewer than 3, the MMRM estimate will not be displayed.

For categorical variables, the proportions will be presented with 95% CIs based on the exact binomial method.

Descriptions and algorithms for some of the efficacy variables are described in Sections 7.2.2.1

7.2.2.1. Clinical Global Impression Scale

The Clinical Global Impression (CGI) scale items 1 (severity of illness) and 2 (global improvement) rating scales are commonly used measures of symptom severity, treatment response and the efficacy of treatments in treatment studies of patients with mental disorders (Busner, 2007).

The Clinical Global Impression - Severity scale (CGI-S) is a 7-point scale that requires the clinician to rate the severity of the patient's illness at the time of assessment, relative to the clinician's past experience with patients who have the same diagnosis. Considering total clinical experience, a patient is assessed on severity of illness at the time of rating as: 1, normal, not at all ill; 2, borderline ill; 3, mildly ill; 4, moderately ill; 5, markedly ill; 6, severely ill; or 7, extremely ill.

The Clinical Global Impression - Improvement scale (CGI-I) is a 7 point scale that requires the clinician to assess how much the patient's illness has improved or worsened relative to a baseline state at the beginning of the intervention and rated as: 1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; or 7, very much worse.

7.2.3. Other Efficacy Analyses

The exploratory endpoint analyses will be conducted on the FA Set.

Additional analyses for the change and percent change from baseline for MELD and Modified Nazer core will be conducted using a mixed model repeated measures (MMRM). Details for calculating MELD (Kamath, 2007; Alcorn, 2015) and Modified Nazer (Dhawan, 2005) can be found in Appendix 9.4.

For the endpoints including improved, stable, and deteriorated categories, these variables are defined in Table 2.

Table 2 Improvement, Deterioration and Stable Categorization

Endpoint	Improve	Deteriorate	Remain Stable

MELD	decrease ≥ 3 points	increase ≥ 3 points	increase or decrease < 3 points
Modified Nazer	decrease ≥ 1 point	increase ≥ 1 point	zero-point change

The proportion of patients in each category will be presented with 95% CIs based on the exact binomial method for all available time points.

For the analyses of the proportion of patients who achieve the MCID, many methods exist for deriving MCID (McDonald, 2013; Yost, 2005; Wyrwich, 1999). MCID values determined from study WTX101-201 will be used in this analysis. The proportion of patients who achieve the MCID will be presented with 95% CI based on the exact binomial method.

A NCC direct assay is in development and should it be validated, the results from the NCC direct assay will be analyzed. Correlations between results from NCC and the NCC direct assay will be summarized. Additionally, should there be sufficient samples of the NCC direct assay for analysis, the primary and secondary efficacy endpoints for NCC may be repeated using the same methodologies described for NCC utilizing the results from the NCC direct assay.

7.3. Safety Analyses

All safety analyses will be conducted on the Safety Set. All safety data will be provided, by treatment cohort, in patient listings. AEs will be coded in MedDRA and presented by MedDRA System Organ Class and Preferred Term. No formal hypothesis testing is planned.

Patients who do not complete the study, for whatever reason, will have all available data up until the time of termination included in the analysis. For safety analysis presented by study visit, the baseline value will be defined as the value reported at the enrollment in this study. The retrospective data prior to the enrollment visit will be used in the case of the value is missing at the enrollment.

The safety endpoints include safety over time, measured by adverse events and safety labs.

7.3.1. Adverse Events (AEs)

Only adverse events (AEs) associated with study related interventional testing or assessments will be collected. Most will be venipuncture AEs. The plan for analysis of AEs described in this section will be performed to the extent of the data available.

Adverse events are recorded on the CRFs. Each AE will be coded to SOC and Preferred Term using MedDRA version 18.0.

For the purposes of this study, an AE is defined as any undesirable physical, psychological or behavioral effect experienced by a subject in conjunction with the blood sample collection procedures described in this protocol for a period of up to 30 minutes post sample collection.

Study displays are described below; additional details are outlined in Appendix 9.4.

7.3.1.1. Overall Summary of Adverse Events

An overall summary of AEs will be presented by treatment cohort and overall, including frequency of patients experiencing the event (n) and relative frequency (n/N*100, where N is the number of patients in the Safety Set). The summary will include categories indicating how many events are AEs and SAEs. Within each category, the following subcategories will also be summarized:

- Toxicity of AEs (Grade 1 through Grade 5)
- AEs leading to death

A listing of all AEs will be presented. Separate listings will be produced for SAEs, AEs resulting in death, and AEs leading to withdrawal from the study.

7.3.1.2. AEs by System Organ Class (SOC) and Preferred Term

The number of AEs and the number and percentage of patients with events will be presented by SOC and Preferred Term. Patients are counted once in each SOC and Preferred Term. Percentages will be based on the total number of treated patients in the treatment cohort. SOCs will be listed in descending frequency as will Preferred Term within each SOC. If needed, terms will also be ordered alphabetically.

SAEs, non-SAEs, and AEs leading to death will be summarized using the same approach.

7.3.1.3. AEs by SOC

The numbers of AEs and the number and percentage of patients with events will be presented by SOC. Patients are counted once in each SOC. Percentages will be based on the total number of treated patients in the treatment cohort.

7.3.1.4. AEs by Preferred Term

The number of AEs and the number and percentage of patients with events will be presented by Preferred Term. Patients are counted once in each Preferred Term. Percentages will be based on the total number of treated patients in the treatment cohort.

7.3.1.5. AEs by SOC, Preferred Term, and Toxicity

The number of AEs and the number and percentage of patients with events will be presented by SOC, Preferred Term and CTCAE grade. If a patient has more than one occurrence of an AE, the highest CTCAE grade reported will be used. If CTCAE grade is missing, the AE will be assumed to be severe (Grade 3). The number of AEs by SOC, Preferred Term and CTCAE grade, without taking into account the highest CTCAE grade, will also be analyzed.

7.3.1.6. Deaths and Other Significant Adverse Events

Any death which resulted in study withdrawal will be reported in disposition tables and listings. Adverse events resulting in death will be presented in a listing by patient. Additionally, listings will be presented for AEs leading to withdrawal from the study.

7.3.1.7. AEs of Special Interest

Not applicable

7.3.2. Other Safety

7.3.2.1. Analyses for Laboratory Tests

All retrospective laboratory measurements will be summarized by visits based on the visit windows defined in Appendix 9.4. All retrospective laboratory data will be included in by-patient data listings. Laboratory measurements will be listed separately by patient, laboratory test, and unit.

Actual values and changes from baseline will be summarized descriptively for patients with available data for each laboratory parameter by treatment cohort and overall. Missing laboratory data will not be imputed and only scheduled assessments will be included in summaries. Multiple records may exist for laboratory parameters with the same date and time and when this occurs, the records will be averaged. A summary for "Last Assessment" will be included for the last available post-baseline result for each patient. A summary of "Worst Post-Baseline" from all post-baseline data will also be included. All data will be included in by-patient data listings. Laboratory measurements will be listed separately by patient, laboratory test, and unit.

Clinical laboratory measurements, including biochemistry, hematology, and coagulation, will be summarized by treatment cohort and all patients combined. Descriptive statistics will be presented for results and changes from baseline at each visit where parameters were scheduled to be collected per the clinical study protocol.

The Investigator will evaluate any out of normal range laboratory values and make a determination as to whether the observation is not clinically significant (NCS) or clinically significant (CS).

Where applicable, laboratory results will be classified as "low," "normal," or "high" with respect to the parameter-specific reference ranges (i.e., below the lower limit of the normal range, within the normal range, or above the upper limit of the normal range). Contingency tables will be presented for each laboratory parameter to summarize the shift from the baseline category to all visits and to the worst post-baseline measurement, defined as the value numerically farthest outside of the normal range across all post-baseline visits through the end of the study.

Summary results will include the count and percentage of patients within each shift category and treatment cohort and all patients combined. Laboratory values outside the normal range will also be summarized and assessed for trends indicating a safety signal. Additionally, a summary and listing of liver enzyme elevation will be presented.

Graphical displays will be provided for the laboratory parameters over time. The retrospective data collected will be shown in the same figure as for the data collected after enrollment.

8. REFERENCES

Alcorn, J.B. (United Network for Organ Sharing). Letter to: Transplant Professionals. 2015 Nov 23. https://optn.transplant.hrsa.gov/media/1575/policynotice_20151101.pdf

Busner, J., Targum, S.D., 2007. The Clinical Global Impressions Scale: Applying a Research Tool in Clinical Practice. Psychiatry (Edgmont) 4(7), 28-37.

Dhawan, A., Taylor, R.M., Cheeseman, P., De Silva, P., Katsiyiannakis, L, Mieli-Vergani, G., 2005. Wilson's Disease in Children: 37-Year Experience and Revised King's Score for Liver Transplantation. Liver Transplantation 11(4), 441-448.

EASL, 2012. European Association for Study of Liver (EASL) Clinical Practice Guidelines: Wilson's disease. J Hepatol 56, 671-685.

Ferenci, P., Caca, K., Loudianos, G., Mieli-Vergani, G., Tanner, S., Sternlieb, I., Schilsky, M., Cox, D., Berr, F., 2003. Diagnosis and phenotypic classification of Wilson disease. Liver international : official journal of the International Association for the Study of the Liver 23, 139-142.

Kamath, P.S., Kim, W.R., 2007. The Model for End-Stage Liver Disease (MELD). Hepatology 45(3), 797-805.

McDonald, C.M., Henricson, E.K., Abresch, T., Florence, J., Eagle, M., Gappmaier, E., Glanzman, A., Spiegel, R., Barth, J., Elfring, G., Reha, A., Peltz, S.. 2013. The 6-Minute Walk Test and Other Clinical Endpoints in Duchenne Muscular Dystrophy: Reliability, Concurrent Validity, and Minimal Clinically Important Differences from a Multicenter Study. Muscle Nerve 48, 357–368.

Morisky, D.E., Green, L.W., Levine, D.M., 1986. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care 24, 67-74.

NCI, 2009. National Cancer Institute Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS). Common Terminology Criteria for Adverse Events V4.0 (CTCAE). Published: May 29, 2009. NIH publication # 09-7473. Available online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. The quick reference guide is available online at: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf, pp.

Plitz, T., 2017. WTX101: Development and validation of NCCcorrected, a method to measure free plasma copper in patients treated with WTX101 or other Cu lowering agents. Wilson Therapeutics AB. Study Number WTX170310. Available upon request.

Wyrwich, K.W., Nienaber, N.A., Tierney, W.M., Wolinsky, F.D., 1999. Linking clinical relevance and statistical significance in evaluating intra-individual changes in health-related quality of life. Med Care, 37, 469–478.

Yost, K.J., Eton, D.T., 2005. Combining distribution- and anchor-based approaches to determine minimally important differences: the FACIT experience. Eval Health Prof, 28, 172–191.

9. APPENDICES

9.1. Protocol Schedule of events

Visit, month	*Enroll*	1	2	3	6	12	18	24
Place of Visit	*Clinic*	*Home[10] or Clinic*	*Home[10] or Clinic*	*Home[10] or Clinic*	*Clinic*	*Clinic*	*Clinic*	*Clinic*
Informed Consent	X							
Eligibility Criteria	X							
Enrollment	X							
Medical History, Incl lab data[1]	X							
Concomitant Medication Including WD Medication History[2]	X							
Blood Sampling (Biochem, Hem, Coag)[3]	X	X	X	X	X	X	X	X
Blood sampling (Cu parameters)[4]	X	X	X	X	X	X	X	X
Blood sampling (PUF)[5]	X	X	X	X	X	X	X	X
Urine Cu parameters[6]	X				X	X	X	X
CGI[7]	X				X	X	X	X
WD Medications[8]					X	X	X	X
Venipuncture AE[9]	X	X	X	X	X	X	X	X

1)The following Medical History is to be collected for up to 5 years prior to study entry if available: Relevant Medical History: WD medical History and Major systemic medical issues. Laboratory data: All available Cu measures, plasma biochemistry [creatinine (including calculated creatinine clearance by Cockcroft-Gault method, blood urea nitrogen (BUN), albumin, protein, total bilirubin, conjugated bilirubin, gamma glutamytransferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), creatinine phosphokinase (CPK)], hematology [Red blood cell count (RBC) (including nucleated RBC), platelets, white cell count (WCC), hemoglobin], and the coagulation measure international Normalized Ratio (INR)

2) Concomitant medication including therapeutic treatment for WD with start date and stop data if applicable, dosage and route, for up to five years prior to study entry if available.

3) Biochemistry: Sodium, potassium, magnesium, chloride, bicarbonate, glucose, urea, creatinine (including calculated creatinine clearance by Cockcroft-Gault method), uric acid, phosphate, total calcium, anion gap, blood urea nitrogen (BUN), cholesterol, albumin, protein, total bilirubin, conjugated bilirubin, gamma glutamyltransferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK). Hematology: Hemogobin, red blood cell count (RBC) (including nucleated RBC), hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, white cell count (WCC), neutrophils, lymphocytes, monocytes, eosinophils and basophils. Coagulation: Prothrombin time (PT), activated partial thromboplastin time (aPTT) and International Normalized Ratio (INR).

4) Plasma: Total Copper (Cu) and Molybdenum (Mo); exchangeable Cu (ExCu); speciation profiling, Ceruloplasmin (Cp) (immune methodology (primary) and enzymatic methodology (exploratory)); on-ceruloplasmin-bound Cu (NCC) (calculated from plasma Cp and total Cu).

5) Plasma Ultrafiltrate (PUF): Cu and Mo (PUF-Cu & PUF-Mo)

6) Urine: 10mL of the routine 24h-urine collection will be extracted for laboratory Cu analysis. Cu and Mo concentrations and volume from 24-hour urine collection to be recorded.

7) Clinical Global Impression item 1 and 2 (at Enrollment visit, item 2 will be 0, 'Not Assessed').

8) Including therapeutic treatments for WD with start date and stop date if applicable, dosage and route
9) AE collection should not include the following:

- AEs that may be associated with the specimen collection procedure but result only in local, mild and transient discomforts, such as hematoma, redness, slight discomfort
- AEs related to the subject WD or treatment, or any other medical condition or event the subject experiences during the study

10) For blood sample collection time points that do not coincide with a routine WD clinic visit, the samples will be drawn, processed and shipped to the testing laboratory either from the WD clinic, or from the subject's home (or subject-specified local address) by a qualifies home health care nurse with experience in obtaining and processing blood specimens for clinical trials.

9.2. Changes from Analyses Specified in the Previous Version of the SAP

This is the first version of the SAP.

9.3. Sample Size, Power, and Randomization

Since the main aim of the study is descriptive, no formal power calculations were performed. In order to capture expected variations in Cu values over time, approximately 60 patients will be included.

9.4. Technical Specifications for Derived Variables

The following derived data will be calculated prior to analysis. The subsections below give more details.

Age

Table 3 Age and reference date

AGE	REFERENCE DATE
• Age at Enrollment	• Date of Signing ICF

Missing date for SoC Treatment

The missing start date will be imputed for SoC treatment according to the following rules.
In cases where only the month and year are provided for a date, the day for the date will be imputed as 15. Missing month will be imputed as June. In cases where both month and day are missing, the date will be imputed as June 15. In instances when the imputed date is earlier than the birth date, the birth date will be used as the reference date.

Baseline Value

Medications and Therapies

Concomitant medications/therapies are any events with administration dates and times on or after the date and time of the baseline visit. If the start date of a medication or therapy is partially or completely missing and the end (stop) date and time of the medication/therapy does not indicate

that it occurred prior to first dose, then the determination of concomitant status will be based on the following:

If the start year is after the year of the first study drug dose, then the medication/therapy is concomitant; else,

if the start year is the same as the year of the first study drug dose and the start month is missing, then the medication/therapy is concomitant; else if the start month is present and is the same or after the month of the first study drug dose, then the medication/therapy is concomitant; else, if the start date is completely missing, then the medication/therapy is concomitant.

All other medications/therapies are considered prior medications/therapies and could occur from the 30 days prior to informed consent up through the Screening Period and prior to the 1st dose.

MELD

Calculate MELD score as follows (Alcorn, 2015):

Candidates who are at least 12 years old receive an initial MELD(i) score equal to:
$MELD(i) = 0.957 \times \ln(Cr) + 0.378 \times \ln(bilirubin) + 1.120 \times \ln(INR) + 0.643$
Then, round to the tenth decimal place and multiply by 10. (Maximum MELD = 40.)

A modification to the MELD score exists for MELD scores greater than 11 (Alcorn, 2015). However, as MELD scores >11 are not expected in this study, it may not be utilized here.

If MELD(i) > 11, perform additional MELD calculation as follows:
$MELD = MELD(i) + 1.32 \times (137 - Na) - [\ 0.033 \times MELD(i) \times (137 - Na)\ ]$

Additional rules:
- All values in US units (Cr and bilirubin in mg/dL, and INR unitless).
- If bilirubin, Cr, or INR is <1.0, use 1.0.
- If any of the following is true, use Cr 4.0:
  - Cr >4.0.
  - ≥2 dialysis treatments within the prior 7 days.
  - 24 hours of continuous veno-venous hemodialysis (CVVHD) within the prior 7 days.
- If Na <125 mmol/L, use 125. If Na >137 mmol/L, use 137.

Modified Nazer Score (Dhawan, 2015)

The score for an individual analyte (bilirubin, AST, INR, white cell count (WCC) and albumin) should be derived from Table 4 and then all 5 scores will be added to get the final score.

Table 4 Modified Nazer Score

Score	Bilirubin (µmol/L)	AST (IU/L)	INR	WCC (10^9/L)	Albumin (g/L)
0	0-100	0-100	0-1.29	0-6.7	>45

1	101-150	101-150	1.3-1.6	6.8-8.3	34-44
2	151-200	151-300	1.7-1.9	8.4-10.3	25-33
3	201-300	301-400	2.0-2.4	10.4-15.3	21-24
4	>301	>401	>2.5	>15.4	<20

Visit Windowing

In analysis of data summarized by study visit, all data collection will be reassigned a study visit where data is scheduled for collection based on the actual days relative to baseline. See Table 5 below for visit windowing that will be used to summarize by-visit efficacy and safety data. Other data will be summarized similarly in accordance to their schedule of events (Appendix 9.1).

Table 5 Visit Windows Used to Summarize by-Visit Efficacy and safety Data

Scheduled Visit	Target Study Day	Study Day Interval
Month -60	-1800	(-1890, -1711)
Month -54	-1620	(-1710, -1531)
Month -48	-1440	(-1530, -1351)
Month -42	-1260	(-1350, -1171)
Month -36	-1080	(-1170, -991)
Month -30	-900	(-990, -811)
Month -24	-720	(-810, -631)
Month -18	-540	(-630, -451)
Month -12	-360	(-450, -271)
Month -6	-180	(-270, -1)
Baseline	1	1
Month 1	30	(2, 44)
Month 2	60	(45, 74)
Month 3	90	(75, 134)
Month 6	180	(135, 269)
Month 12	360	(270, 449)
Month 18	540	(450, 629)
Month 24	720	(630, 930)

If more than one value is mapped to the same scheduled visit, the closer of those values will be considered for summarization. Visit windows are intended to be contiguous such that all data collected at all post-baseline visits, whether scheduled or unscheduled, will map to one of the visits. Table 5 is also meant to display the general pattern for how all other parameter visit windows will be determined and can be summarized with the following formula: study day interval lower bound = target study day – ((target study day – last target study day)/2) rounded down to the nearest integer.

The visit displayed on subject data listings will be reflective of the scheduled visit label as reported on the eCRF. Study days relative to baseline will be displayed for each visit so it is apparent which visit the data may have been reassigned to in the summaries.

9.5. Additional details on Statistical Methods

Not applicable.

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Signer Events	Signature	Timestamp
PPD		Sent: 03-Jun-2019 | 14:31 Viewed: 03-Jun-2019 | 14:32 Signed: 03-Jun-2019 | 14:38

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PPD		Sent: 03-Jun-2019 | 14:38 Viewed: 03-Jun-2019 | 14:48 Signed: 03-Jun-2019 | 14:54

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In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	03-Jun-2019 | 14:38
Certified Delivered	Security Checked	03-Jun-2019 | 14:48
Signing Complete	Security Checked	03-Jun-2019 | 14:54
Completed	Security Checked	03-Jun-2019 | 14:54

Payment Events	Status	Timestamps