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

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Study Director: PPD
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Statistician: PPD

The clinical trial protocol amendment 1 has been reviewed and approved by the Sponsor:

Wilson Therapeutics Signature PPD Date: 21 Sept. 2016

Printed Name: PPD

Title: PPD

CONFIDENTIAL INFORMATION

PROTOCOL AMENDMENT 1 SIGNATURE PAGE

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

This protocol is sponsored by Wilson Therapeutics AB (Sponsor). All data and any other proprietary information arising from the execution of this study remain the sole property of Wilson Therapeutics and may not be used for any purpose without Wilson Therapeutics prior written permission.

This study will be conducted in accordance with International Conference on Harmonisation (ICH) guidelines for Good Clinical Practices (GCPs), the Basic Principles of the Declaration of Helsinki, and local ethical and legal requirements.

Investigator's Statement:

I have read and understood the information in this protocol and agree to conduct the trial according to the protocol (subject to any amendments). Any changes to the protocol must be approved by Wilson Therapeutics prior to seeking approval from the Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Amendments to the protocol must be approved by the IRB or IEC.

I agree to conduct in person or to supervise the trial.

I agree to ensure that all that assist me in the conduct of the study are aware of their obligations.

Investigator's Signature: _____

Printed Name: _____

Name of Institution/Company: _____

Date: _____

Please submit a signed copy of this page to Wilson Therapeutics.

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1 PROTOCOL SYNOPSIS

TITLE:

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

PROTOCOL NO:

WTX101-203

INVESTIGATOR/ STUDY CENTERS:

Up to 15 centers in North America and Europe.

OBJECTIVES:

The primary objective of the study is to assess plasma and urine Copper (Cu) parameters in Wilson Disease (WD) subjects treated with Standard of Care (SOC) medications.

The secondary objective is to compare Cu parameters with corresponding clinical data, including medical and medication history, clinical laboratory results, Wilson Disease (WD) medications, and Clinical Global Impression (CGI).

METHODOLOGY:

This is a 24-month study to assess Cu parameters in subjects with WD treated with SOC medications. Data will be collected during routinely scheduled WD clinic visits at approximately 6-month intervals. Data collected will include: relevant medical history and WD medication history (including all Cu measurements and relevant clinical laboratory results for up to five years prior to study enrollment, if available), concomitant medications for up to five 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 24h-urine collection for assessment of Cu parameters, creatinine clearance, calculated modified Nazer score, WD medications, and subjects 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 will be collected for analysis at the central laboratory at 8 time points (Enrollment, 1, 2, 3, 6, 12, 18, and 24 Months), i.e. at 5 routine clinic WD visits and at 3 additional time points.

After providing informed consent, subjects meeting all inclusion and no exclusion criteria will be enrolled into the study as outpatients. Subject's routine WD clinic visits will be 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 do not coincide with a routine WD clinic visit, the samples will be 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 will be required for subject participation in this protocol.

At the time of enrollment, subjects will be receiving SOC therapeutic agents for the treatment of WD. If treatment is interrupted or stopped during the course of the study, subjects will continue in

the study and biological samples and clinical data will continue to be 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, will be 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 will be performed by the site local laboratory as determined by the treating physician per the standard practice for the management of subjects with WD.

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

The central laboratory will be contracted by Wilson Therapeutics. Results from the central laboratory testing will be available to, but will not be used by, the treating physicians for the clinical management of study subjects. Central laboratory results will be 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) will be entered into a web-based electronic data capture (EDC) system by the study physician or study coordinator/designee. The modified Nazer score will be calculated by a program in the EDC system for each of the visits. Wilson Therapeutics will be responsible to contract with and manage the EDC system vendor.

Wilson Therapeutics or its designee will be responsible for writing the clinical study report.

NUMBER OF SUBJECTS:

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

STUDY DURATION:

Subjects will participate for approximately 24 months after signing the informed consent.

INVESTIGATIONAL DRUG:

This is not an investigational drug study. At the time of enrollment, subjects will be receiving SOC agents as prescribed by the treating physician and according to clinical practices at the study center. This may include penicillamine, trientine, zinc, or a combination of a Cu chelator and zinc. Dosing with SOC agents will be prescribed/individualized by the treating physician at the study center according to standard clinical practice at the site.

DATA FOR COLLECTION/EVALUATION:

Laboratory Values:

- Copper parameters
    - Plasma: Total Copper (Cu) and Molybdenum (Mo); exchangeable Cu (ExCu);

speciation profiling, Ceruloplasmin (Cp) (immune methodology will be the primary methodology and an enzymatic methodology will be tested as an exploratory method); non-ceruloplasmin-bound Cu (NCC)

- Plasma Ultrafiltrate (PUF): Cu and Mo (PUF-Cu & PUF-Mo)
- Urine: Cu and Mo concentrations and volume from 24-hour urine collection

- Biochemistry, hematology, coagulation:
  Hepatic measures (including alanine aminotransferase (ALT), aspartate transaminase (AST), International Normalized Ratio (INR) and total and conjugated bilirubin), and routine chemistry, hematology, coagulation testing and creatinine clearance.

Other Clinical Data:

- Medical and Medication history, WD medications, Clinical Global Impression (CGI) scale (item 1 and 2), and modified Nazer score.

INCLUSION/EXCLUSION CRITERIA:

Subjects must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Willing and able to give informed consent for participation in the study.
2. Male or female subjects, aged 18 years or older as of signing the ICF.
3. Receiving Standard of Care therapeutic agents (penicillamine, trientine, zinc, or copper chelators with zinc) for the treatment of Wilson Disease at the time of enrollment and for no more than 60 months prior to enrollment.
4. Able to understand and willing to comply with study procedures and requirements, as judged by the Investigator.
5. Established diagnosis of Wilson Disease.
6. Adequate venous access to allow for collection of blood samples.

Subjects will be excluded from this study if they meet any of the following criteria:

1. Major systemic disease or other illness that would, in the opinion of the Investigator, compromise subject safety or interfere with the collection or interpretation of study results.
2. In the opinion of the Investigator, the subject is likely to be non-compliant or uncooperative during the study.

STATISTICAL METHODS:

The main statistical analyses will be descriptive. No formal hypothesis testing is planned. The main analysis is to describe and display copper parameters measured by a central laboratory over time. This will be done both graphically and by summary statistical measures such as mean, median, and standard deviation over time.

2 ABBREVIATIONS

aPTT	activated partial thromboplastin time
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CBC	complete blood count
CFR	Code of Federal Regulations
CGI	Clinical Global Impression
Cp	ceruloplasmin
CRF	case report form
Cu	copper
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
ExCu	Exchangeable copper
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
INR	International Normalized Ratio
IRB	Institutional Review Board
LDH	lactate dehydrogenase
MCH	mean cell hemoglobin
MCHC	mean cell hemoglobin concentration
MCV	mean cell volume
mg	milligram
mL	milliliter
Mo	molybdenum

MT	metallothionein
NCC	non-ceruloplasmin-bound copper
PI	Principal Investigator
PT	prothrombin time
PUF	plasma ultrafiltrate
PUF-Cu	plasma ultrafiltrate copper
PUF-Mo	plasma ultrafiltrate molybdenum
RBC	red blood cell
SAP	statistical analysis plan
SEC	size exclusion chromatography
SOC	Standard of Care
US	United States
WCC	white blood cell count
WD	Wilson Disease

3 INTRODUCTION

3.1 Background

Wilson Disease (WD) is a rare autosomal recessive disorder caused by mutations in the ATP7B gene resulting in deficient production of the copper (Cu)-transporter Adenosine Triphosphatase 2 (ATPase2), which in turn leads to impaired incorporation of Cu into Ceruloplasmin (Cp) as well as impaired biliary excretion of Cu. Consequently, there is an increase of Cu in liver, brain, and other tissues. Beyond the capacity of metallothionein (MT) to bind and buffer Cu, the excessive amount of intracellular free Cu triggers pro-oxidant properties leading to organ damage and dysfunction (Pfeiffer, 2007). The overall prevalence of WD is estimated at 1-2 in 30,000, corresponding to approximately 10,000 – 20,000 individuals in the US (Frydman, 1990; Reilly *et al.*, 1993; Schilsky, 2002) (FDA Orphan Drug Designation: 11-3465).

The clinical presentation of WD typically occurs in adolescence to early adulthood. Genetic screening and genotype-phenotype correlation is complicated by a multitude of associated *ATP7B* mutations; most individuals with WD are compound heterozygotes. Initial signs and symptoms of WD are hepatic (~40%), neurological (~40%) or psychiatric (~20%) although patients often develop combined hepatic and neuropsychiatric symptoms. Approximately 10% are asymptomatic when diagnosed with WD. Untreated or inadequately treated patients have progressive morbidity, and mortality is usually secondary to hepatic cirrhosis. Liver transplantation is the only effective therapy for WD-associated acute liver failure. Other causes of death associated with WD include hepatic malignancy and neurological deterioration with severe inanition (Pfeiffer, 2007; Roberts and Schilsky, 2008).

The treatment goals in WD are to reduce Cu to normal levels and then maintain these levels. The current treatments for WD are chelator therapies D-penicillamine and trientine, which non-specifically chelate Cu and promote urinary Cu excretion. In addition, zinc, which blocks dietary uptake of Cu, is used mainly for maintenance treatment. Zinc impairs the absorption of Cu by the induction of MT in the gastrointestinal tract.

3.2 Unmet Medical Need

Currently available drugs for WD have high rates of treatment discontinuation due to adverse events (AE). They also need to be dosed 2-4 times per day and must be taken in the fasted state. Their AE profiles and complicated dosing regimens leads to poor treatment compliance and high rates of treatment failure, a major concern with a chronic disease that requires lifetime treatment such as WD (Maselbas *et al.*, 2010).

Disease control in patients with neurological symptoms at WD diagnosis is an area of particular concern. In a recent overview of WD, patients with a neurological presentation demonstrated improvements in neurological aspects of their disease in only 67.5% of the patients treated with D-penicillamine and only 55% of the patients treated with trientine (Weiss *et al.*, 2013). Also, in a recent study, as many as approximately 50% of patients had residual neurological symptoms despite years of therapy on a de-coppering agent (Holscher *et al.*, 2010). Worsening of neurological symptoms can also occur on initiation of treatment

due to mobilization of Cu from the liver leading to spikes in blood Cu associated with neurological progression (Brewer *et al.*, 2009).

3.3 Rationale for the WTX101-203 Standard of Care Study

Total plasma free Cu, which is considered to mediate Cu toxicity in WD, could be assessed through non ceruloplasmin-bound copper (NCC), but NCC has limited value in the clinic due to high variability. Cu control in WD patients is commonly monitored indirectly through analysis of 24-hour Cu excretion in urine.

In addition to the commonly used 24h-urine Cu excretion and NCC, this protocol will assess Cu parameters via further measures, i.e. ExCu and PUF-Cu. The correlation of these blood Cu measures with the clinical presentation of the patient is currently insufficiently understood. It is therefore expected that the data on direct Cu control measurements and free Cu obtained in this study will facilitate monitoring of Cu control and aid the management of patients with WD.

Such data may also facilitate the evaluation of new and existing therapies for the WD. Wilson Therapeutics is developing an investigational de-coppering agent, WTX101 (bis-choline tetrathiomolybdate), for treatment of WD. WTX-101 targets several medical needs, including control of Cu by the formation of stable Cu-tetrathiomolybdate-protein complexes leading to a rapid de-coppering without mobilizing free Cu that could cause tissue toxicity, including neurological deterioration. This investigational agent will not be used in this study. However, as Cu-tetrathiomolybdate-protein complexes are not primarily excreted in the urine, the availability of test methods that are able to provide direct Cu control monitoring data would potentially facilitate the evaluation of this investigational drug.

This protocol is designed to assess Cu parameters in biological samples from subjects 18 years of age or older receiving SOC treatments for WD. In addition to analyses of Cu parameters in biological samples, corresponding clinical data is collected, including medical and medication history, retrospective clinical laboratory results, WD medications, and treating physician impression of overall clinical status. These data will be used to generate additional knowledge on Cu dynamics in WD patients receiving SOC therapy, and to optimize the design of future clinical trials that will assess the safety and efficacy of WTX101 for the treatment of WD.

4 OBJECTIVES

4.1 Primary Objective

The primary objective of the study is to assess plasma and urine Copper (Cu) parameters in Wilson Disease (WD) subjects treated with Standard of Care (SOC) medications.

4.2 Secondary Objectives

The secondary objective is to compare Cu parameters with corresponding clinical data, including medical and medication history, clinical laboratory results, Wilson Disease (WD) medications, and Clinical Global Impression (CGI).

5 INVESTIGATIONAL PLAN

5.1 Overall Study Design

This is a 24-month study to assess Cu parameters in subjects with WD being treated with SOC medications. Data will be collected during routinely scheduled WD clinic visits at approximately 6-month intervals. Data collected will include relevant medical history and WD medication history (including all Cu measurements and relevant clinical laboratory results for up to five years prior to study enrollment if available), concomitant medication for up to five 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 24h-urine collection for assessment of Cu parameters, WD medications, and subjects 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 will be collected for analysis at the central laboratory at 8 time points (Enrollment, 1, 2, 3, 6, 12, 18, and 24 Months), i.e. at 5 routine clinic WD visits and at 3 additional time points.

After providing informed consent, subjects meeting all inclusion and no exclusion criteria will be enrolled into the study as outpatients. Subject's routine WD clinic visits will be 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 blood sample collection time points at Month 1, 2 and 3 that do not coincide with a routine WD clinic visit, the samples will be 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 at each of the 8 blood collection time points, no other interventional assessment will be required for subject participation in this protocol.

At the time of enrollment, subjects will be receiving SOC therapeutic agents for the treatment of WD for not more than 60 months. If treatment is interrupted or stopped during the course of the study, subjects will continue in the study and biological samples and clinical data will continue to be 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, will be 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 will be performed by the site local laboratory as determined by the treating physician per the standard practice for the management of subjects with WD.

In parallel with the local laboratory, a central laboratory will be also be utilized for the measurement of laboratory values, including Cp, total Cu and Mo, exchangeable Cu, speciation profiling, PUF-Cu and PUF-Mo, and routine chemistry, hematology, and coagulation testing. The samples collected for the central laboratory testing will be used to

assess the Cu and WD related parameters, and the correlation of these parameters with corresponding clinical information.

The central laboratory will be contracted by Wilson Therapeutics. Results from the central laboratory testing will be available to, but will not be used by, the treating physicians for the clinical management of study subjects. Central laboratory results will be 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) will be entered into a web-based electronic data capture (EDC) system by the study physician or study coordinator/designee. The modified Nazer will be calculated by a program in the EDC system. Wilson Therapeutics will be responsible to contract with and manage the EDC system vendor.

Wilson Therapeutics or its designee will be responsible for writing the clinical study report.

6 STUDY POPULATION

6.1 Study Entry Criteria

6.1.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Willing and able to give informed consent for participation in the study.
2. Male or female subjects, aged 18 years or older as of signing the ICF.
3. Receiving 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.
4. Able to understand and willing to comply with study procedures and requirements, as judged by the Investigator.
5. Established diagnosis of Wilson Disease.
6. Adequate venous access to allow collection of blood samples.

6.1.2 Exclusion Criteria

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

1. Major systemic disease or other illness that would, in the opinion of the Investigator, compromise subject safety or interfere with the collection or interpretation of study results.
2. In the opinion of the Investigator, the subject is likely to be non-compliant or uncooperative during the study.

6.2 Subject Discontinuation or Termination

Subjects may be discontinued from study for the following reasons:

1. Investigator considers it would be in the best interests of the subject
2. Subject requests discontinuation for any reason
3. Subject is unable or unwilling to comply with the protocol.

7 STUDY SCHEDULE AND BLOOD SAMPLE AND DATA COLLECTION

7.1 Study visits

7.1.1 Enrollment visit (Baseline)

All subjects must provide written informed consent before undergoing any study-related procedures. The Principal Investigator (PI), or designee, will explain to the subject the aims of the study, the risks and benefits involved and the fact that their participation is voluntary. Each subject will acknowledge receipt of this information and that they wish to partake in the study by giving written informed consent for their involvement in the study in the presence of the PI, or designee, who will also sign and date the Informed Consent Form (ICF). Time, date, name of the person taking consent, and any questions raised by the subject should be documented in the source data.

The following data will be collected and entered into the EDC system for the Enrollment Visit:

- Date of Visit
- Date ICF was signed
- Inclusion and Exclusion Criteria review/confirmation
- Demographic information: sex, date of birth, ethnicity
- Relevant Medical History (including WD medical history and major systemic medical issues, all available Cu information, available relevant clinical laboratory measurements and normal ranges, current and for up to five years prior to study entry if available)
- Concomitant Medications including WD Medication History (with start date and stop date if applicable, dosage and route, current and for up to five years prior to study entry if available)
- Blood sampling and processing time for central laboratory analysis of Cu parameters
- Blood sampling and processing time for central laboratory analysis of biochemistry, hematology and coagulation measures
- Blood sampling and processing time for PUF-Cu and PUF-Mo for central laboratory analysis
- Urine for Cu parameters (10 mL of the routine 24h-urine collection will be extracted for central laboratory analysis, total urine volume must be noted)
- Overall impression of subject assessed by CGI scale item 1 (item 2 will always be given the value 0, for not assessed. See section 7.4 for CGI description)
- Any AEs related to study specific blood draw (with the exception of AEs that result only in local, mild and transient discomforts, such as hematoma, redness, slight discomfort). For details, see section 10.

7.1.2 Non-SOC, Study specific visits performed either at WD clinic or subject specified location

Visits at Month 1, 2 and 3 after the Enrollment visit are expected not to coincide with routine clinical care visits. For these visits, subjects will be offered the option to have the samples

drawn at the WD clinic or at their homes (or a subject-specified local address) by a qualified home health care nurse with experience in obtaining and processing blood specimens for clinical trials. No blood samples will be drawn for analysis at the local laboratory at any of these visits.

The following data will be collected and entered into the EDC system for the visits outside routine clinical care:

- Date of Visit
- Blood sampling and processing time for central laboratory analysis of Cu parameters
- Blood sampling and processing time for central laboratory analysis of biochemistry, hematology and coagulation measures.
- Blood sampling and processing time for PUF-Cu and PUF-Mo for central laboratory analysis
- Any AEs related to study specific blood draw (with the exception of AEs that result only in local, mild and transient discomforts, such as hematoma, redness, slight discomfort). For details, see section 10.

7.1.3 Routine SOC WD clinic visits

The following data will be collected and entered into the EDC system for the subjects routine SOC clinic visits, expected at approximately 6 months intervals following Enrollment visit:

- Date of Visit
- Blood sampling and processing time for central laboratory analysis of Cu parameters
- Blood sampling and processing time for central laboratory analysis of biochemistry, hematology and coagulation measures
- Blood sampling and processing time for PUF-Cu and PUF-Mo for central laboratory analysis
- Urine for Cu parameters (10 mL of the routine 24h-urine collection will be extracted for central laboratory analysis, total urine volume must be noted)
- Overall impression of subject assessed by CGI scale item 1 and 2 (see section 7.4 for CGI description)
- WD Medication (with start date and stop date if applicable, dosage and route)
- Any AEs related to study specific blood draw (with the exception of AEs that result only in local, mild and transient discomforts, such as hematoma, redness, slight discomfort). For details, see section 10.

7.1.4 Early Termination Visit

There is no requirement for an early termination visit for subjects who choose to withdraw from the study or are asked by the Investigator or Sponsor to discontinue study participation.

7.2 Study Specific Biological Sample Collection

As described in the visit paragraphs above, and in Section 8 below, study specific blood samples will be collected at the following 8 time points during the study: Enrollment, 1, 2, 3, 6, 12, 18 and 24 Months. The blood samples for the protocol-specified central laboratory analysis equals a total amount of up to 240 mL, and are collected in addition to the blood samples collected for local laboratory testing ordered by the treating physician for the SOC management of WD.

No other study specific biological samples are collected. For central laboratory urine Cu analyses, 10 mL of the 24h-urine collection that is performed as part of routine management of WD patients is extracted.

7.3 Retrospective clinical laboratory measurement collection

Laboratory data and normal ranges, 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.

The following clinical laboratory measurements are considered relevant for this protocol:

Biochemistry: Creatinine (including calculated creatinine clearance by Cockcroft-Gault method), blood urea nitrogen (BUN), albumin, protein, total bilirubin, conjugated bilirubin, gamma glutamyltransferase (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).

7.4 Clinical Global Impression (CGI)

Overall impression of subject's condition is assessed at every routine clinic visit by the Clinical Global Impression (CGI) scale.

At Enrollment visit (baseline) first item is assessed, while second item will always be 0. At visits planned approximately 6, 12, 18 and 24 Months after enrollment, item 1 and 2 of the scale are assessed.

The items of CGI scale are as follows:

- Item 1. Considering your total clinical experience with this particular population, how ill is the patient at this time?

  Severity Score:
  0 Not assessed

1 Normal, not at all ill
2 Borderline ill
3 Mildly ill
4 Moderately ill
5 Markedly ill
6 Severely ill
7 Extremely ill

- Item 2. Compared to the subject's condition at baseline, how much has he/she changed?

Improvement Score
0 Not assessed
1 Very much improved
2 Much Improved
3 Minimally Improved
4 No change
5 Minimally worse
6 Much worse
7 Very much worse

Baseline for this assessment is the enrollment visit into this study.

8 CENTRAL LABORATORY ANALYSES

8.1 Clinical Laboratory Measures

8.1.1 Central laboratory analyses of biochemistry, hematology and coagulation

The central laboratory will perform the following testing:

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: Hemoglobin, 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).

8.1.2 Central laboratory analyses of copper parameters

The central laboratory will perform the following testing of Cu parameters:

- *Plasma copper (Cu) and molybdenum (Mo)*
  - Total Cu and Mo
  - Exchangeable Cu (ExCu)
  - Speciation profiling to evaluate Cu and Mo containing molecular species using SEC
- *Plasma ceruloplasmin (Cp)*
  - Cp will be measured by immune-methodology (primary analysis) and by enzymatic methodology (exploratory assay)
  - Non-ceruloplasmin-bound Cu (NCC) will be calculated from plasma Cp and total Cu values
- *Plasma Ultrafiltrate (PUF)*
  - Cu and Mo (PUF-Cu and PUF-Mo)
- *Urine Cu and Mo*
  Cu and Mo concentrations and volume from the 24-hour urine collection

8.1.3 Central laboratory sample processing

Wilson Therapeutics will provide a central laboratory manual that will detail the handling of all study samples to be analyzed by the Wilson designated central laboratory. Sites will follow their own local laboratory procedures for samples being tested at the site.

9 STATISTICAL METHODS

9.1 Statistical Analyses

The main statistical analyses will use descriptive methods. No formal hypothesis testing is planned. The main analysis is to describe and display the change in Cu parameters over the course of the study. This will be done both graphically and by summary measures such as mean, median and standard deviation over time.

Baseline values and patient characteristics will be presented in tables by center and in total. Continuous variables will be summarized using standard statistical measures, i.e. number of subjects, mean, standard deviation, median, minimum, maximum. Categorical variables will be summarized in frequency tables.

Since the subject's change in Cu parameters is important, historical data for the previous five years if available is collected. Hence, trends in Cu parameters before inclusion in the study can be assessed. By studying both historical and follow-up data the statistical analyses will provide valuable information regarding the natural variation in Cu values.

9.2 Sample size

Since the main aim of the study is descriptive no formal power calculation has been performed. In order to capture expected variations in Cu values over time approximately 60 subjects will be included.

10 SAFETY

This is not an investigational drug study. With the exception of providing 8 blood samples above and beyond those required for subjects SOC therapy, subjects will not undergo any other study-related interventional testing or assessments.

The procedures outlined in this protocol do not pose known significant risk to subject safety. Subjects will be provided with the investigator's contact information and will be instructed to notify the investigator of any Adverse Events (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.

The following AEs will **not** be collected in 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 event the subject experiences during the study.

The treating physician is responsible to monitor the safety of subjects who are receiving SOC medications for WD and reporting this information if/as needed to the appropriate regulatory authorities according to local and/or country regulations and standard clinical practice at the study sites.

11 STUDY ADMINISTRATION

The names, titles, and addresses of the Investigators and study personnel are listed in the Site Contacts list in the Study Reference Manual for Protocol WTX101-203 and are available from Wilson Therapeutics.

11.1 Ethical Considerations

This research will be carried out in accordance with the protocol, US Code of Federal Regulations, GCP, 21 CFR Parts 50, 56, and 312, the ethical principles set forth in the Declaration of Helsinki, and the ICH harmonized tripartite guideline regarding GCP (E6 Consolidated Guidance, April 1996).

11.1.1 Informed Consent Form

A sample ICF will be provided to each site. No major deviations may be made from the sample ICF other than country- or region-specific formatting or requirements. Wilson Therapeutics or its designee will review the draft ICF before it is finalized, and the final IRB/EC-approved documents must be provided to Wilson Therapeutics for regulatory purposes.

The ICF must be signed by the subject or the subject's legal representative before the subject can participate in the study. The signature of an impartial witness will be accepted only in the case that the subject is not able to provide the signature by him/herself because of physical impairments. In this case, the subject must be deemed mentally able by the PI to understand the study and able to can give their informed consent verbally. A copy of the signed ICF must be provided to the subject or the subject's legal representative. If applicable, it will be provided in a certified translation of the local language.

The original signed ICF must remain in each subject's study file and must be available for verification by study monitors.

11.1.2 Institutional Review Board or Independent Ethics Committee Approval

This protocol, the ICF, relevant supporting information and all types of subject recruitment or advertisement information must be submitted to Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) for review and must be approved in writing before the study is initiated. Any amendments to the protocol must also be approved, where necessary, by the IRB/IEC prior to implementing changes in the study.

The Investigator is responsible for keeping the IRB/IEC apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once a year as per applicable regulations. The Investigator must also keep the IRB/IEC informed of any AEs, according to the IRB/IEC policy and/or local regulations.

11.2 Study or Site Termination

If Sponsor, an Investigator, or regulatory authorities discover conditions during the study that indicate that the study or related activities at a particular site should be terminated, this action may be taken after appropriate consultation between Sponsor and the Investigator. Conditions that may warrant study or site termination include but are not limited to:

1. Data recording is inaccurate or incomplete
2. Investigator(s) do not adhere to the protocol or applicable regulatory guidelines in conducting the study
3. GCP is not being maintained or adequately followed
4. Administrative reasons
5. Reasons unrelated to the study

Study or site termination and follow-up will be performed in compliance with the conditions set forth in 21 Code of Federal Regulations (CFR) Section 3.1.2 and/or other national and local regulations, as applicable, and in compliance with the principles set forth in International Conference on Harmonisation (ICH) Good Clinical Practices (GCPs) and ethical principles established by the Declaration of Helsinki.

11.3 Study Documentation and Recordkeeping

Monitoring and auditing procedures developed by Wilson Therapeutics will be followed in order to comply with ICH Good Clinical Practice (GCP) guidelines. On-site review of the CRFs for completeness and clarity, cross checking with source documents, and clarification of administrative matters will be performed. Monitoring will be done by personal visits from representatives of Wilson Therapeutics (CRAs/site monitors). Monitoring visits will consist of periodic visits during the study period and a site close-out visit as outlined in a study-specific Monitoring Plan.

The Investigator will permit authorized representatives of Wilson Therapeutics and the respective national or local authorities to inspect facilities and records relevant to this study.

11.4 Quality Assurance

The study will be initiated and conducted under the sponsorship of Wilson Therapeutics. Data collected for the study will be recorded in the eCRFs and source documents. Representatives of Wilson Therapeutics will monitor the study to verify study data, medical records, worksheets, and CRFs are completed in accordance with current International Conference on Harmonisation (ICH) GCPs and the respective local and national government regulations and guidelines.

11.5 Confidentiality

The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted below is prohibited.

Information obtained during the conduct of this study will be collected, processed, and transmitted to Wilson Therapeutics in accordance with the applicable regulations and principles of confidentiality for each participating country. Information contained therein will be maintained in accordance with applicable law protecting patient privacy, including the provisions of 46 CFR Part 164 promulgated under the Health Insurance Portability and Accountability Act (HIPAA) and may be inspected by the clinical researcher, the researcher's staff, Sponsor and its representatives, partners, advisors, affiliates, successors, and clinical research contractors and subcontractors to check, process, evaluate, and use the information collected during the study. The subject ICF will be used to obtain participant consent to authorize transfer and processing of data consistent with applicable law.

To ensure confidentiality, after signing the written informed consent, each subject will be assigned a unique study identification number. The first two digits will correspond to a pre-assigned Study Site number. The second two digits will correspond to the sequential order in which each subject is enrolled at that particular Study Site. (e.g., the first subject enrolled at the first site = 01-01, first subject enrolled at the second site = 02-01, etc.). The Subject ID numbers will be used to record subject study data into EDC and Central Laboratory databases.

Information obtained from the study will likely be used by Wilson Therapeutics or its affiliates or successors in connection with the development of study drug, including possible filing of applications with governmental authorities for marketing approval, and for other pharmaceutical and medical research purposes. The study Investigator is obliged to provide Sponsor with access to complete test results and data collected this study. This information may be disclosed to applicable regulatory authorities as deemed necessary by Wilson Therapeutics. Subject-specific information may be provided to other appropriate medical personnel only with the subject's permission, as necessary and in accordance with other applicable privacy laws and regulations protecting subject health information.

To ensure compliance with the ICH GCP guidelines, data generated by this study must be available for inspection upon request by representatives of the appropriate national and local authorities, Wilson Therapeutics, and the IRB/EC for each study site.

Wilson Therapeutics may actively pursue publication of the results of the study in cooperation with the PIs subject to the terms and conditions of the clinical trial agreement between Wilson Therapeutics and Investigators.

11.6 Protocol Adherence

Each Investigator must adhere to the protocol as detailed in this document and agree that any changes to the protocol must be approved by a Wilson Therapeutics authorized representative in writing prior to seeking approval, where necessary, from the IRB or IEC. Each

Investigator will be responsible for allowing only those subjects who have met all protocol eligibility criteria to be enrolled.

Modifications to the protocol should not be made without agreement of the Investigators and Wilson Therapeutics. Changes to the protocol will require written IRB or IEC approval / favorable opinion prior to implementation. The IRB/IEC may provide expedited review and approval/favorable opinion for minor change(s) in ongoing trials that have the approval/favorable opinion of the IRB/IEC. The Investigator will submit all protocol modifications to the IRB/IEC in accordance with the governing regulations.

11.7 Source Documentation

The Investigator must maintain detailed records of all study participants who are enrolled in the study. Source documents include subject medical records and Investigator's subject study files. Information required for study purposes and any data recorded in the eCRF must be supported by appropriate source documentation.

11.7.1 Direct Access to Source Documentation

The Investigator will ensure that the Sponsor, IRB, and regulatory authorities will have direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing (ICH[E6] 5.1.2 & 6.10). The Investigator will contact the Sponsor immediately if contacted by a regulatory agency about an inspection at his or her center. The purpose of Sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ICH/GCP guidelines, and any applicable regulatory requirements.

11.8 Clinical Study Report

Wilson Therapeutics will provide the final study report.

11.9 Case Report Forms

An electronic data capture system (EDC) will be provided to each investigational site for the collection of all study data for enrolled subjects in an eCRF, with the exception of data that may be captured in an electronic format (i.e., central laboratory data). Study site personnel will record the data in the source documentation and enter it on the CRF within, on average, 5 business days of the subjects WD clinic follow-up visits, while carefully reviewing all information recorded for accuracy and consistency. Any required data printouts should be filed in the subject's source data, i.e., laboratory reports, etc.

The CRFs for each subject must be reviewed and signed by the Investigator. This should be done as soon as possible after the subject has completed the study and all data queries have been resolved. A clinical study monitor will review the CRFs and compare the content to the source data.

11.10 Training of Staff

The PI is responsible for the conduct of the study at this study site, including delegation of specified study responsibilities, and training of study staff. The PI shall ensure that the study is carried out in accordance with the protocol, ICH/GCP guidelines, and regulations.

11.11 Retention of Records

Records and documents pertaining to the conduct of this study, including CRFs, source documents, consent forms, laboratory test results, and medication inventory records, must be retained by the Investigator in accordance with locally applicable regulatory requirements. No study records shall be destroyed without notifying Sponsor and giving Sponsor the opportunity to take such study records or authorizing in writing the destruction of records after the required retention period.

12 REFERENCES

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13 SCHEDULE OF STUDY 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 glutamyltransferase (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 treatments for WD with start date and stop date 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**: Hemoglobin, 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)); non-ceruloplasmin-bound Cu (NCC) (calculated from plasma Cp and total Cu).

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

6) Urine: 10 mL 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 qualified home health care nurse with experience in obtaining and processing blood specimens for clinical trials.