Alexion Pharmaceuticals, Inc.

# INTERIM STATISTICAL ANALYSIS PLAN
## For Regulatory Submission of Ongoing Study

PROTOCOL NUMBER: ALXN1840-WD-204

# A PHASE 2, OPEN-LABEL STUDY TO ASSESS COPPER AND MOLYBDENUM BALANCE IN PARTICIPANTS WITH WILSON DISEASE TREATED WITH ALXN1840

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**Date:** 08 Dec 2021

**Version:** 2.0

# 1. APPROVAL SIGNATURES

[redacted]

Date dd Mmm yyyy

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# 3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and acronyms are used in this statistical analysis plan (SAP).

**Table 1: Abbreviations and acronyms**

| Abbreviation or acronym | Explanation |
|---|---|
| $\lambda_z$ | apparent terminal-phase elimination rate constant |
| ADME | absorption, distribution, metabolism, and excretion |
| AE | adverse event |
| ALT | alanine aminotransferase (SGPT) |
| AR | accumulation ratio |
| AST | aspartate aminotransferase (SGOT) |
| ATC | Anatomical Therapeutic Chemical |
| AUC | area under the plasma concentration versus time curve |
| $AUC_t$ | AUC from time 0 to the last quantifiable concentration |
| $AUC_{tau}$ | AUC over the dosing interval |
| $AUC_\infty$ | AUC from time 0 to infinity |
| AUEC | area under the effect versus time curve |
| $AUEC_t$ | AUEC from the start of dose administration to the last observed quantifiable concentration |
| BLQ | below the limit of quantification |
| BMI | body mass index |
| $CE_{max}$ | maximum observed effect after dosing |
| CI | confidence interval |
| CL/F | apparent total body clearance |
| $C_{max}$ | maximum observed concentration |
| Cp | ceruloplasmin |
| CpC | ceruloplasmin-bound copper |
| CRF | case report form |
| CRU | clinical research unit |
| $C_\tau$ | observed concentration at the end of the dosing interval |
| CTCAE | Common Terminology Criteria for Adverse Events |
| $C_{trough}$ | trough (pre-dose) concentration observed at the start of the dosing interval |
| CV | coefficient of variation |
| ECG | electrocardiogram |
| EOS | end of study |
| FA | Full Analysis |
| GGT | gamma glutamyltransferase |
| GM | geometric mean |
| ICF | informed consent form |
| ICH | International Conference on Harmonization |
| ICP-MS | inductively coupled plasma mass spectrometry |
| INR | international normalized ratio |
| I/O | input/output |
| LBC | labile bound copper |
| LLOQ | lower limit of quantification |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MMRM | mixed model repeated measures |
| NCC | non-ceruloplasmin-bound-copper |

| Abbreviation or acronym | Explanation |
|---|---|
| $NCC_{corrected}$ | non-ceruloplasmin-bound copper concentration corrected for the amount of copper bound to the copper-tetrathiomolybdate-albumin tripartite complex formed after ALXN1840 administration |
| NCI | National Cancer Institute |
| PD | pharmacodynamic(s) |
| PK | pharmacokinetic(s) |
| PP | Per Protocol |
| PT | Preferred Term (MedDRA) |
| PTAE | pre-treatment adverse event |
| PUF | plasma ultrafiltrate |
| QT | interval between the start of the Q wave and the end of the T wave |
| QTc | QT interval corrected |
| QTcF | QT interval corrected using Fridericia's formula |
| SAE | serious adverse event |
| SAS® | Statistical Analysis Software® |
| SAP | statistical analysis plan |
| SD | standard deviation |
| SoA | schedule of activities |
| SOC | system organ class (MedDRA) |
| $t_{1/2}$ | terminal elimination half-life |
| TEAE | treatment-emergent adverse event |
| $TE_{last}$ | time after dosing at which the last quantifiable concentration was observed |
| $TE_{max}$ | time after dosing at which the maximum effect was observed |
| $T_{lag}$ | time delay between the time of dosing and time of appearance of molybdenum concentration |
| $T_{last}$ | time of last quantifiable concentration |
| $T_{max}$ | time to maximum concentration |
| ULOQ | upper limit of quantification |
| UNS | unscheduled |
| $V_d/F$ | apparent volume of distribution |
| WD | Wilson Disease |
| WHO | World Health Organization |

# 4. DESCRIPTION OF THE PROTOCOL

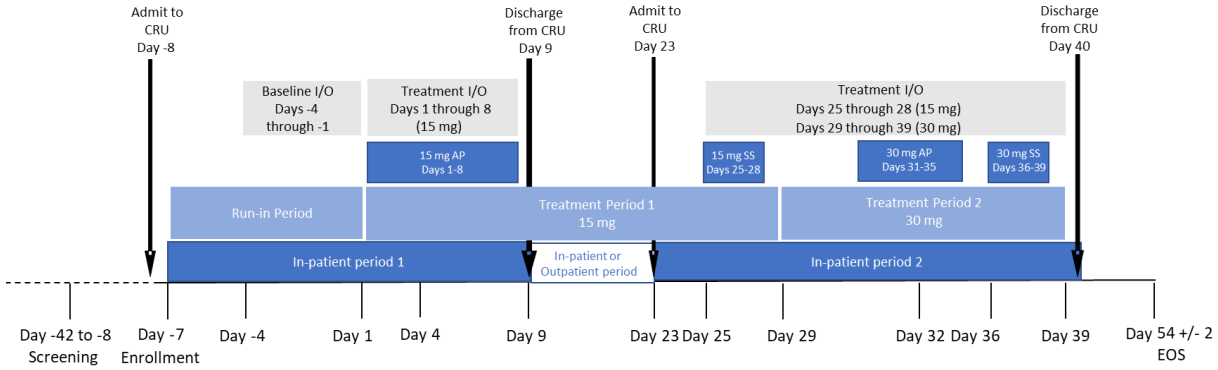
Study ALXN1840-WD-204 will be conducted as an open-label, repeat-dose study to evaluate the effects of ALXN1840 on copper balance in participants with Wilson Disease (WD).

Treatment-experienced (which includes standard of care therapies or ALXN1840) and treatment-naïve participants are eligible for this study. Eligible patients will be classified into one of two cohorts:

- Cohort 1 (treatment experienced): Patients who have received WD therapy for > 28 days
- Cohort 2 (treatment naïve): Patients who have received WD therapy for ≤ 28 days

Following Screening and enrollment, participants will check-in to the clinical research unit (CRU) on Day -8 for the Run-in Period. The purpose of the Run-in Period is to support diet equilibration (Day -7 through Day -5) and measure pretreatment copper and molybdenum balance (Day -4 through Day -1). Participants will remain on a copper/molybdenum-controlled diet throughout both the Inpatient Period 1 (Day -8 to Day 9) and Inpatient Period 2 (Day 23 to Day 40). The study design schematic in presented in Figure 1. While not in the CRU, participants will be encouraged to adhere to their usual copper-controlled diet.

**Figure 1: Study Design Schematic**




1. Participants eligible for the study will be enrolled on Day -7 and initiated on a copper- and molybdenum-controlled diet, which will be continued during Treatment Period 1 and Treatment Period 2 when in the CRU, through Day 39.
2. Participants will be admitted to the CRU for Treatment Period 1 on Day -8 and may be discharged on Day 9 or remain in the CRU for their own safety or to maintain the integrity of the conduct of the study. Participants who are discharged will be re-admitted on Day 22 or Day 23 for Treatment Period 2 and will remain in house until Day 40. To ensure flexibility, the Outpatient Period may be extended up to an additional 14 days with Investigator approval. In this situation, participants will be given additional study drug to support daily dosing throughout the Outpatient Period. In such cases, the actual Outpatient Period duration will be recorded and the participant will continue Inpatient Period 2 at Day 23.
3. Participants will be administered ALXN1840 at a dose of 15 mg/day on Day 1 through Day 28 and then increase to 30 mg/day on Day 29 through Day 39.
4. Participants will have 3 intake and output collection periods including:
   - Baseline Day -4 through Day -1
   - Day 1 through Day 8 (Input/Output (I/O) for 15 mg)
   - Day 25 through Day 39 (Days 25 through 28, I/O for 15 mg; Days 29 through 39, I/O for 30 mg)

5. During these periods all intake (including food and drink) and output (including urine and feces) will be collected and assessed for copper and molybdenum.

Abbreviations: AP = accumulation period; EOS = End of Study; SS = steady state.

Participants who are taking copper-chelating therapies (penicillamine or trientine) at the time of enrollment will be discontinued from their decoppering therapies starting on Day -4 to allow a Baseline assessment of copper/molybdenum balance prior to ALXN1840 treatment. On Day 1, participants will be initiated on 15 mg/day of ALXN1840 for a Treatment Period of approximately 28 days followed by titration up to 30 mg/day on Day 29. Before titration to 30 mg/day, the SRC will review available safety data through Day 23 for each participant.

Participants will have intake and output collection periods from Day -4 through Day -1, from Day 1 through Day 8, and Day 25 through Day 39. The collection periods will support an assessment of both copper and molybdenum balance (as a measure of ALXN1840 absorption, distribution, metabolism, and excretion [ADME]) at the 15 mg and 30 mg doses and will allow assessment of the effects of duration of treatment on copper elimination and copper balance.

Collection periods for feces and urine will vary in duration from 3 to 15 days to support assessment of both copper and molybdenum balance before and at steady state for both 15 mg and 30 mg. Equilibration periods on copper/molybdenum-controlled diets will be a minimum of 48 hours. Copper balance will be calculated as the mean daily copper balance over each of the 4 collection periods. The interpretation of copper balance will be based on the criteria established by Hill (1986) when undertaking copper balance studies with zinc treatment. For assessment of ALXN1840 effect on copper balance, the time period for analysis will take into consideration the average bowel transit of approximately 40 hours (male: 33 hours; female: 47 hours) (Camilleri, 1986; Metcalf, 1987).

Throughout the inpatient periods, participants will remain on a copper-controlled diet. Meal portions will be weighed and meal sizes will be appropriate to support male and female caloric consumption. Participants will be encouraged to complete 100% of all meals throughout the inpatient period to support quantification of copper and molybdenum intake. If participants are unable to complete the full meal, the uneaten portion will be weighed to allow calculation of meal fraction, and the copper and molybdenum intake will be adjusted based on the fractional intake of the meal. In addition to food, fluid intake and type will be measured and recorded each day. Similarly, if participants do not complete 100% of non-water fluids with meal, the remaining volume will be measured and recorded. Samples of all meals and fluids will be sent for bioanalysis to support accurate quantification of copper and molybdenum in fluids. In the event that items cannot be accurately quantified, items may be balanced during pre-Treatment Period and post-Treatment Period to support the change from Baseline assessment.

During the inpatient collection periods, daily urine will be pooled (24-hour collection) with volumes recorded for each 24-hour period; participants will be strongly encouraged to void within 2 hours of completion of each 24-hour period (ie, dosing time). Stool samples will be individually collected and each sample will include a collection date, time, and weight.

Participants may be discharged from the CRU on Day 9 and return on Day 22 or Day 23 (pre-dose); all procedures will start on Day 23. To ensure flexibility, the Outpatient Period during Treatment Period 1 may be extended up to an additional 14 days with Investigator approval. In this situation, participants will be given additional study drug to support daily dosing throughout the Outpatient Period. In such cases, the actual Outpatient Period duration will be recorded and

the participant will continue Inpatient Period 2 at Day 23. During the Outpatient Period, participants will use a study dosing diary to record study intervention administration. At the CRU's discretion, participants may remain in the CRU during the outpatient period for safety or to maintain the integrity of the conduct of the study.

Blood sampling for pharmacokinetic (PK)/pharmacodynamic (PD) will occur over the 24-hour dosing period on Days 1, 25, 29, and 39. Pre-dose PK samples will be collected at all time points during the intake and output collection period to help characterize PK during accumulation (Days 1 through 9 for 15 mg and Days 31 through 35 for 30 mg) and at steady state (Days 25 through 28 for 15 mg and Days 36 through 39 for 30 mg).

This study incorporates the use of an adaptive design. Adaptive features may be implemented at the discretion of the Investigator to support conduct of the study. Such adaptive features do not require amendment of the protocol. Adaptive features and their limits are described in the Protocol Table 4.

The overall objective of this Interim Statistical Analysis Plan (SAP) is to describe the second and subsequent analysis of interim data from Study ALXN1840-WD-204 to support the ALXN1840 regulatory submissions.

## 4.1. Changes From Analyses Specified in the Protocol

Not applicable. There are no changes from the protocol.

## 4.2. Changes From Analyses Specified in the Previous Version of the Statistical Analysis Plan

The following updates have been made to the SAP version 1.0 approved 11 Jun 2021:

- Added analyses for WD history. (Section 7.1.3.3)
- Added analyses for prior medications used to treat WD. (Section 7.1.4)
- Added language to clarify that sensitivity analysis will only be performed when the number of participants in the Per Protocol Set is at least 3. (Section 7.2.1.5)
- Added analyses for Cu and Mo input, output, and input to output sources on a percentage basis. (Section 7.2.3).

# 5. DEFINITIONS

## 5.1. Efficacy

### 5.1.1. Primary Objective and Endpoint

The primary objective is to demonstrate a net negative copper balance with daily repeat-dose ALXN1840 treatment (15 mg and 30 mg) in participants with WD.

The primary endpoint is the mean daily copper balance where copper balance is measured by the calculated difference between copper intake (in food and drink) and copper output (in feces and urine) during ALXN1840 accumulation and steady-state periods for each dose.

#### 5.1.1.1. Balance Measurements

Copper balance measurements will be made on all intake (ie, study drug, food and fluids) and all output (urine and feces) from participants as indicated in the schedule of activities (SoA) in the Protocol Table 1. The copper concentration of each sample will be determined by inductively coupled plasma mass spectrometry (ICP-MS). Copper content of all intake and output will be calculated based on the volume or weight of intake and output and the concentration of representative samples.

##### 5.1.1.1.1. Food and Fluid Collection for Copper and Molybdenum Concentrations

Samples of all meal and fluid batches will be collected and analyzed for measurement of copper content. A minimum of 3 complete portions/meals from each food and liquid batch will be sent for analysis. All participants will drink water from the same large water bottle dispenser. Samples of water from this dispenser will be collected and analyzed for copper content.

Samples will be collected, stored and shipped as detailed in the Laboratory Manual. All sample handling procedures will be documented in detail in the Laboratory Manual. Copper concentration of each food sample (ng/g) and each fluid sample (ng/mL) sample will be determined by ICP-MS.

##### 5.1.1.1.2. Urine Collection for Measurement of Copper and Molybdenum Content

Urine samples to measure copper content will be collected periodically as described in the SoA (Protocol Table 1). Samples will be collected, stored and shipped as detailed in the Laboratory Manual. For each 24-hour collection period, urine will be pooled for analysis and volumes will be recorded. All sample handling procedures will be documented in detail as described in the Laboratory Manual. Copper concentration (ng/mL) of each 24-hour urine sample will be determined by ICP-MS.

##### 5.1.1.1.3. Fecal Collection for Measurement of Copper and Molybdenum Content

Fecal samples to measure copper content will be collected periodically as described in the SoA (Protocol Table 1). Samples will be collected, stored, and shipped as detailed in the Laboratory Manual. Fecal samples will be individually collected, weighed, and stored. The weight and time of each bowel movement will be recorded. All sample handling procedures will be documented

in detail in the Laboratory Manual. Copper concentration (ng/g) of each stool sample will be determined by ICP-MS; each sample will be analyzed using a minimum of technical triplicates.

### 5.1.2. Secondary Objectives and Endpoints

The secondary objectives and endpoints are given below. Molybdenum balance measurements are conducted in a similar manner to copper balance measurements (Section 5.1.1.1).

| Objectives | Endpoints |
|---|---|
| Assess change in copper balance in response to ALXN1840 15 mg/day and 30 mg/day during ALXN1840 accumulation and at steady state periods versus the pretreatment Baseline in participants with WD | Change in mean daily copper balance as measured by the calculated difference between copper intake (in food and drink) and copper output (in feces and urine) from pretreatment Baseline (Days -4 through -1) and ALXN1840 accumulation and steady-state periods for each dose |
| Investigate the effect of ALXN1840 (15 mg/day and 30 mg/day) on the disposition of copper in participants with Wilson Disease (WD) | Copper quantified in food, drink, feces, and urine, including plasma total and labile bound copper (LBC) during ALXN1840 accumulation and steady-state periods for each dose |
| Investigate the effect of ALXN1840 on the disposition of molybdenum at steady state at 15 mg/day and 30 mg/day in participants with WD | Molybdenum quantified in food, drink, feces, and urine, plasma total molybdenum at ALXN1840 steady state |
| Assess steady-state total molybdenum balance as a measure of ALXN1840 15 mg/day and 30 mg/day in participants with WD | Mean daily molybdenum balance as demonstrated through measurement of molybdenum intake (in food, drink, and ALXN1840), and molybdenum output (feces and urine) representing ALXN1840 steady state |
| Assess accumulation of molybdenum with ALXN1840 treatment at 15 mg/day and 30 mg/day in participants with WD | Accumulation of molybdenum as determined by molybdenum balance |
| Determine the steady-state plasma pharmacokinetic (PK) of total molybdenum and plasma ultrafiltrate (PUF) molybdenum (as surrogate measures of ALXN1840, 15 mg and 30 mg) in participants with WD | PK parameters for plasma total and PUF molybdenum |

Note: The accumulation period refers to time from initiation of ALXN1840 (Day 1) to expected steady state at Day 10, based on 5 times the half-life of 2 days.

### 5.1.3. Exploratory Objectives and Endpoints

The exploratory objectives and endpoints are:

| Objectives | Endpoints |
|---|---|
| Determine dose response of ALXN1840 15 mg/day and 30 mg/day for copper balance in participants with WD | Assess dose response of ALXN1840 on copper balance focusing on copper balance |
| Determine the effect of treatment duration on copper balance in participants with WD | Determine the effect of time following initiation of ALXN1840 treatment on copper balance |
| Assess the effects of ALXN1840 on ceruloplasmin (Cp), ceruloplasmin-bound copper (CpC), LBC profiles in plasma in participants with WD | Cp, CpC, LBC, and Change in Cp at Days 1, 8, 25, 29, 36, and Day 39 compared with pre-dose |
| Assess dose proportionality at steady state of doses of ALXN1840 15 mg/day and 30 mg/day in participants with WD | Based on PK parameters |

| Objectives | Endpoints |
|---|---|
| Assess effects of ALXN1840 on copper:molybdenum ratio in plasma at steady state in participants with WD | Measure plasma copper:molybdenum ratios at steady state compared with pre-dose |
| Assess the effects of repeat-dose ALXN1840 on copper:molybdenum ratio in urine and feces in participants with WD | Mean daily copper:molybdenum ratio in urine and feces at steady state compared with pre-dose Baseline |

## 5.2. Safety

The safety objective of this study is to evaluate the safety and tolerability of repeated-dose administration of ALXN1840 15 mg/day and 30 mg/day in participants with WD.

Treatment emergent adverse events (TEAEs)/serious adverse events (SAEs), clinical laboratory assessments (serum chemistry, hematology, coagulation, and urinalysis), and physical examinations will be evaluated. Heart rate, intervals (PR, QRS, interval between the start of the Q wave and the end of the T wave (QT) and QT interval corrected using Fridericia's formula (QTcF)), clinically significant electrocardiogram (ECG) findings as determined by triplicate 12-lead ECG, and vital sign assessments (blood pressure and heart rate) will also be assessed. The SoA is provided in Table 1 of the Protocol.

### 5.2.1. Adverse Events

The definitions of adverse events (AEs) and SAEs can be found in Section 10.3 of the Protocol.

All AEs will be reported to the Investigator or qualified designee by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

All AEs and SAEs will be collected from the signing of the informed consent form (ICF) until the End of Study (EOS) Visit.

Suicidal ideation and behavioral changes will be recorded as adverse events and may, at the discretion of the Investigator, result in withdrawal of the participation from the study and urgent referral for psychiatric treatment.

Further details are given in the Protocol Section 8.3 and Protocol Appendix 3.

### 5.2.2. Laboratory Assessments

Clinical chemistry, hematology, coagulation, urinalysis and other tests will be performed.

Participants' bowel movements and urination will be monitored by the clinical staff. The clinical staff will record in the case report form (CRF) each time a fecal and urine sample is collected.

To support accurate quantification of copper/molybdenum intake, each participant's intake including both food and fluids will be monitored and recorded. Following each standardized meal, the clinical staff will record 100% completion of each meal including all liquids. If a participant is unable to eat 100% of the food for a given meal, the remaining food will be weighed and reported in the CRF to support accurate determination of copper/molybdenum as a fraction of the total meal. Similarly, if participants do not complete 100% of non-water fluids with meal, the remaining volume will be measured and recorded in the CRF. The staff will record daily water volume intake in the CRF.

Refer to Protocol Section 10.2 for the list of clinical laboratory tests.

### 5.2.3. Vital Signs

Vital signs will be measured in a supine position after 5 minutes rest and will include body temperature, respiratory rate, systolic and diastolic blood pressure, and heart rate.

### 5.2.4. Physical Examinations

A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, and neurological systems. Height (at Screening only) and weight (as per the SoA for physical examinations) will also be measured and recorded. A symptom-driven physical examination may be performed at other times, at the Principal Investigator's discretion.

### 5.2.5. Electrocardiograms

Triplicate 12-lead ECGs will be conducted as outlined in the SoA to obtain heart rate, PR, QRS, QT, and QTc intervals corrected using Fridericia's formula (QTcF). As with vital signs, if ECG interval measurements are abnormal, an additional triplicate will be performed and recorded in the CRF.

## 5.3. Pharmacokinetics/Pharmacodynamics

### 5.3.1. Pharmacokinetics

Whole blood samples will be collected for the measurement of plasma concentrations of total molybdenum and PUF molybdenum as specified in the SoA (Protocol Table 1) via ICP-MS. Samples collected within ± 10% or 30 min, whichever is less, of the scheduled time will not be considered a protocol deviation.

The actual date and time (24-hour clock time) of each sample will be recorded.

Total molybdenum and PUF-molybdenum are surrogate measures of ALXN1840 and concentrations will be used to evaluate the PK of ALXN1840. Samples collected for analyses of plasma concentrations may also be used to evaluate safety aspects related to concerns arising during or after the study.

### 5.3.2. Pharmacodynamics

Plasma total copper, PUF copper, ceruloplasmin (Cp), ceruloplasmin-bound copper (CpC), and LBC will be assessed during the study.

Blood samples will be collected as described in the SoA (Protocol Table 1) for plasma isolation as per the Laboratory Manual. Plasma samples will be used for ICP-MS measurement of total copper, PUF copper, Cp, CpC, and non-ceruloplasmin-bound copper measured via PUF copper, and/or LBC, or assessed via non-ceruloplasmin-bound-copper (NCC)/$NCC_{corrected}$ methods at the time points indicated in the SoA.

# 6. DATA SETS ANALYZED (STUDY POPULATIONS)

For this interim analysis, the database will be cleaned through a specific date, and the database will include only data through that date (the data-cut date). The date of the data-cut for this interim analysis will be provided in the clinical study report (CSR).

## 6.1. Screened Set

All participants who signed the ICF.

## 6.2. Enrolled Set

All participants who signed the ICF, were eligible for the study, and were registered on Day -7 when participants are assigned a participant number.

## 6.3. Full Analysis Set

All participants who received at least 1 dose of ALXN1840 treatment.

## 6.4. Per Protocol Set

All participants who received at least 1 dose of ALXN1840 treatment, had Baseline and all post-Baseline values of copper intake (in food and drink) and copper output (in feces and urine), and were 100% compliant with study drug dosing. Participants with important protocol deviations that are likely to impact the primary endpoint analysis will be excluded from the Per Protocol (PP) Set. Important protocol deviations and the PP Set will be defined, documented, and agreed within Alexion prior to database lock.

## 6.5. Safety Set

All participants who received at least 1 dose of ALXN1840.

## 6.6. Pharmacokinetic Analysis Set

All participants who have sufficient plasma samples to enable the calculation of PK parameters and provide PK profiles.

## 6.7. Pharmacodynamic Analysis Set

All participants who have sufficient plasma samples to enable the calculation of PD parameters and provide PD profiles.

# 7. STATISTICAL ANALYSIS

Summary statistics will be computed and displayed by visit where applicable and will be presented by cohort (treatment experienced/treatment naïve), and overall. Descriptive statistics for continuous variables will minimally include the number of participants, mean, standard deviation (SD), minimum, median, and maximum. For categorical variables, frequencies and percentages will be presented. If the number of participants is deemed to be too small, descriptive statistics might not be presented and only listings will be presented. Graphical displays will be provided as appropriate.

Analyses will be performed using the Statistical Analysis Software® (SAS®) Version 9.4 or higher (SAS Institute Inc., Cary, North Carolina).

## 7.1. Study Participants

### 7.1.1. Disposition of Participants

The number and percentage of all participants screened, enrolled, included in the Full Analysis (FA), Per-Protocol (PP), Safety, PK, and PD analysis sets will be summarized. The reasons for exclusion from the analysis sets will also be provided. Frequency counts and percentages of participants excluded prior to enrollment will be provided for participants who failed to meet study entry requirements during Screening.

The number and percentage of participants who completed, or prematurely discontinued from the study will be summarized by cohort and overall. For participants who discontinued the study, the number and percentage will be summarized by their reason for premature discontinuation. Additionally, the number of participants who completed or prematurely discontinued treatment and the reason for treatment discontinuation will be summarized. A summary of participants who did not meet inclusion or who met exclusion criteria will be provided.

Descriptive statistics of the number of days in the study will be summarized. The date of first and last use of the study drug, and the study termination date will be listed. Individual reasons for premature discontinuation from the study will be presented in a listing, as will the reason for premature discontinuation from study treatment. All enrolled participants will be listed indicating their membership to each analysis set along with the reason for exclusion. Additionally, a listing of the inclusion/exclusion criteria and a listing of participants and the inclusion criteria they failed to meet and the exclusion criteria they met will be provided. A listing of screen failure participants will also be provided.

### 7.1.2. Protocol Deviations

All important/not important protocol violations will be determined and appropriately categorized prior to database lock. The number and percentage of participants with any important/not important protocol violations as well as the number and percentage of participants with violations within each category will be presented. A listing will also be provided.

### 7.1.3. Demographics, Disease Characteristics, and Medical History

All demographic and Baseline characteristics information will be summarized on the FA Set. Summary statistics will be presented by cohort and overall. Continuous variables will be

presented using descriptive statistics, and categorical variables will be presented using frequencies and percentages. Age will be calculated relative to signing of informed consent and will be summarized as both a continuous and categorical variable. Listings will also be provided.

#### 7.1.3.1. Demographics

The following demographic variables will be summarized:

- Sex
- Race (where collection is permitted by local regulations)
- Ethnicity (where collection is permitted by local regulations)
- Age (years)
- Height (cm)
- Weight (kg)
- Body mass index (BMI) ($kg/m^2$)

#### 7.1.3.2. Baseline Disease Characteristics

The following Baseline characteristics will be summarized:

- Time since WD diagnosis
- Cumulative duration of prior WD treatment
- Cirrhosis
- LBC (µmol/L)
- LBC categorization based on normal reference range (low: <0.7 µmol/L for female, <0.9 µmol/L for male; normal: 0.7 to 5.9 µmol/L for female, 0.9 to 4.4 µmol/L for male; high: >5.9 µmol/L for female, >4.4 µmol/L for male)
- Calculated NCC (µmol/L)
- NCC categorization based on normal reference range (<0.8 µmol/L, 0.8 to 2.3 µmol/L, >2.3 µmol/L)
- Directly measured NCC (µmol/L)
- Albumin (g/L)
- International normalized ratio (INR)
- Total bilirubin (µmol/L)
- Direct bilirubin (µmol/L)
- Alanine aminotransferase (ALT) (U/L)
- Aspartate aminotransferase (AST) (U/L)
- Gamma glutamyltransferase (GGT) (U/L)

- Platelets ($10^9$/L)
- Leukocytes ($10^9$/L)
- Creatine kinase (U/L)
- Hemoglobin (g/L)
- Total plasma copper (µmol/L)
- Total plasma copper categorization based on normal reference range (low: < 11.33 µmol/L [< 720 ng/mL]; normal: 11.33 - 26.12 µmol/L [720 - 1660 ng/mL]; high: > 26.12 µmol/L [> 1660 ng/mL])
- PUF copper (µmol/L)
- CpC (µmol/L)
- Total plasma molybdenum (µmol/L)
- PUF molybdenum (µmol/L)
- Cp (µmol/L)
- 24-hour urinary copper (µg/day)
- 24-hour urinary molybdenum (µg/day)
- Categorization of Baseline lab values (ALT, AST, GGT, platelets, leukocytes, creatine kinase) based on the reference ranges (L: Low, N: Normal, H: High)

#### 7.1.3.3. Medical/Surgical History, and WD History

Medical and surgical history will be summarized by counts and percentages and displayed by System Organ Class (SOC) and Preferred Term (PT) within each SOC. Both SOCs and PTs will be coded using Medical Dictionary for Regulatory Activities (MedDRA), Version 22.0 or higher, available at the start of the study. This dictionary will be used throughout the life of the study and will not be updated during the study conduct. The number and percentage of participants will be presented for ongoing conditions and previous conditions separately by SOC and PT. A listing will also be created.

All details of WD diagnosis will be listed in full.

### 7.1.4. Prior and Concomitant Medications/Therapies

Prior medications will be defined as medications that were discontinued prior to the start of study drug. Concomitant medications will be defined as medications that either started prior to first dose of study drug and were continuing at the time of first dose of study drug or started on or after the date of the first dose of study drug. If it cannot be determined whether a medication was stopped prior to the start of study drug dosing due to partial or missing medication start or end dates, it will be considered a concomitant medication.

The World Health Organization (WHO) Drug Dictionary version from March 2018 or later will be used to code the medications. Medications will be summarized by Anatomical Therapeutic Chemical (ATC) level 3 class and generic drug name.

Prior and concomitant medications will be summarized separately for the FA Set by cohort and overall. The number and percentage of participants receiving any concomitant medication will be summarized, as well as the number and percentage receiving any concomitant medication by ATC drug class and generic drug name. Participants reporting use of more than 1 medication at each level of summarization (any medication received, ATC class, and generic drug name) will be counted only once. All ATC class terms will be displayed alphabetically and generic drug names within each ATC class will be displayed by descending order of incidence. Prior medications used to treat WD and an additional analysis of all prior medications will be summarized similarly. WD treatment history will be summarized by counts and percentages for Cohort 1 and Cohort 2 by prior WD medication, where prior WD medication is defined as the later of the treatment at the time of Screening or most recent treatment prior to Screening and is categorized as penicillamine, trientine hydrochloride, zinc, or combination.

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

## 7.2. Efficacy Analyses

The analyses and summaries will be based on the FA Set. For by-visit analyses, the definition is given in Appendix 9.2. Listings will also be provided for all efficacy assessments.

### 7.2.1. Primary Analysis

Based on the ALXN1840 mechanism of action, the primary objective of this study is to demonstrate a net negative copper balance with daily repeat-dose ALXN1840 treatment (15 mg and 30 mg) in participants with WD.

The primary analysis will be performed using the FA Set. Average daily copper balance and molybdenum balance will be calculated over the following periods:

- Day -4 through Day -1 representing pre-dose Baseline
- Days 1 through 8 representing the ALXN1840 15 mg/day accumulation period
- Days 25 through 28 representing the ALXN1840 15 mg/day steady-state period
- Days 31 through 35 representing the ALXN1840 30 mg/day accumulation period
- Days 36 through 39 representing the ALXN1840 30 mg/day steady-state period

Copper balance is defined by the difference in copper input and copper output. A negative Copper balance will indicate greater copper output than copper intake. Copper input is defined as the sum of all copper input as measured in all food and fluids over the specified period. Copper output is defined as the sum of all copper output as measured in urine and feces over the specified collection period. Many of the copper inputs and outputs will be collected with technical replicates and the mean of the replicates will be utilized in the analysis. The average daily copper balance is calculated as the difference in copper input and copper output during the specified period, divided by the total number of days in that period.

As ALXN1840 is expected to increase copper excretion through fecal excretion, copper in stool will be critical for determining copper balance. Because the timing of bowel movements can be irregular, assessment of copper and molybdenum balance will only include data up to the day of

the final bowel movement. For example, if the participant does not have a bowel movement on Day 8, then average daily copper balance for the Day 1 through Day 8 period will only include data from Day 1 through Day 7.

In the case of the 15 mg/day steady-state period (ie, Day 25 through Day 28), stool data collected on Days 29 - 30 samples may be used if needed to support assessments for the 15 mg/day dose. Use of these stool data (as needed) are consistent with an approximately 2-day gastrointestinal transit time.

In the case of stool irregularity, and to support assessment of copper output over time, bowel movement copper and molybdenum outputs may be averaged over the days between bowel movement (or start of study) to ensure an approximate value for each 24-hour period.

The daily copper balance for each subject will be plotted as will the mean daily copper balance.

#### 7.2.1.1. Handling of Dropouts or Missing Data

Subjects who prematurely discontinue from the study will be included up to the time of discontinuation.

#### 7.2.1.2. Subgroup Analysis

There is no planned subgroup analysis.

#### 7.2.1.3. Multicenter Studies

Not applicable.

#### 7.2.1.4. Hypothesis Testing and Significance Level

As this study is an exploratory study, no formal hypothesis testing is being conducted. The study results will be summarized using descriptive statistics.

#### 7.2.1.5. Sensitivity Analyses

As a part of sensitivity analysis, the primary analysis in Section 7.2.1 will also be performed on the PP Set.

Sensitivity analysis will only be performed when the number of participants in the PP Set is at least 3.

### 7.2.2. Secondary Analyses

Secondary analyses will be performed using the FA Set. The secondary endpoints include:

- Change from pre-dose baseline in mean daily copper balance during ALXN1840 accumulation and steady-state periods for each dose
- Mean daily copper quantified in food, drink, feces, and urine, and mean plasma total copper, and mean LBC, during ALXN1840 accumulation and steady-state periods for each dose
- Mean daily molybdenum quantified in food, drink, feces, and urine, and mean plasma total molybdenum, during ALXN1840 steady-state period for each dose

- Mean daily molybdenum balance as demonstrated through measurement of molybdenum intake (in food, drink and ALXN1840), and molybdenum output (feces and urine), during ALXN1840 steady-state period for each dose
- Accumulation of molybdenum as determined by molybdenum balance
- PK parameters for plasma total and PUF-molybdenum

The secondary continuous endpoints will be analyzed using the same methods described for the primary analysis. Molybdenum balance will include the additional molybdenum intake from ALXN1840. The nominal amount of molybdenum contained in ALXN1840 will be used and is estimated as 3.33 mg in each 15 mg tablet per the following calculation: 95.96 g/mol (molecular weight of molybdenum)/ 432.54 g/mol (molecular weight of bis-choline tetrathiomolybdate) * 15 mg/tablet = 3.33 mg molybdenum. The periods of interest described in Section 7.2.1 will be utilized as appropriate. The daily molybdenum balance for each subject will be plotted as will the mean daily molybdenum balance.

### 7.2.3. Exploratory Analyses

The exploratory endpoint analyses will be conducted on the FA Set. The exploratory endpoints will be analyzed in the same manner as the primary endpoint. Additionally, plasma copper:molybdenum ratio, copper:molybdenum ratio in urine and feces, and ratio of cumulative Cu and Mo Inputs and Outputs will be examined. The ratios will be derived at the patient level daily and then summarized. The periods of interest described in Section 7.2 will be utilized as appropriate.

Descriptive statistics for treatment period 1 (15 mg) and treatment period 2 (30 mg) for the net copper balance will be presented to understand the dose response relationship. A boxplot with jitter scatterplot will be included to show the net copper balance in the two dose groups.

## 7.3. Safety Analyses

Safety analyses will be performed using the Safety Analysis Set.

Safety analyses will include all AEs, ECGs, clinical laboratory data, physical examinations, and vital sign measurements using descriptive statistics.

No inferential statistical analyses are planned for the safety parameters of this study.

### 7.3.1. Study Duration, Treatment Compliance, and Exposure

Study duration will be summarized for all enrolled participants by cohort and overall. Study duration is defined as the time from first dose to the EOS or study discontinuation date (whichever occurs first) + 1.

Compliance during the Inpatient and Outpatient periods will be determined as the days taken / the days expected to be taken during active study treatment x 100. Treatment compliance will be summarized using descriptive statistics within cohort and overall. If a subject prematurely discontinues, his or her compliance will be based on the period up to the point of discontinuation from the study. Compliance will also be summarized using counts and percentages by compliance category (ie, ≥ 100% compliance, 80 to <100% compliance, 60 to <80% compliance, etc.). A supporting listing will also be produced.

Exposure will be summarized for the Safety Set by cohort and overall. The duration (days) of exposure to treatment for each period will be calculated as date of last exposure to treatment – date of first dose + 1. The actual duration of treatment will be calculated as the exposure period – number of days on which treatment was temporarily stopped. The duration of exposure, average daily dose (mg), minimum daily dose (mg) and maximum daily dose (mg) for each period (as described in Section 7.2.1), and overall, as applicable will be summarized using descriptive statistics. Listings of exposure, drug interruptions and missed doses will also be presented.

### 7.3.2. Adverse Events

The verbatim terms as reported in the CRF by Investigators to identify AEs will be coded using MedDRA Version 22.0 or higher and summarized by primary SOC and PT.

Adverse event toxicity will be evaluated using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (published 27 Nov 2017).

Adverse event causality is determined by the Investigator using the following assessment categories: unrelated, or related.

Treatment-emergent AEs are defined as those AEs with onset on or after the first dose of treatment (ie, study drug). Events reported with a partial onset date (eg, month and year are reported but the day is missing) will be considered to be treatment-emergent if it cannot be confirmed that the event onset was prior to the first dose of study drug based on the available date entries.

#### 7.3.2.1. Overall Summary of Adverse Events

An overall summary of TEAEs will be presented, including number of events and number and percentage of participants experiencing AEs. Percentage will be calculated as n/N*100, where n is the number of participants with events and N is the number of participants in the Safety Set. The summary will include categories indicating how many events are TEAEs, treatment-emergent SAEs, and treatment-emergent non-SAEs. Within TEAEs, the following subcategories will also be summarized:

- Toxicity of TEAEs (Grade 1 through Grade 5)
- Related TEAEs (not related, related)
- TEAEs leading to withdrawal of study drug
- TEAEs leading to withdrawal from the study
- TEAEs leading to death

A summary of events (E) and number of participants with events (n, %) for pre-treatment emergent events (PTAEs) will also be included with its relevant subcategories.

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

#### 7.3.2.2. Adverse Events and Serious Adverse Events by System Organ Class and Preferred Term

The number of TEAEs and the number and percentage of participants with events will be presented by SOC and PT. Participants are counted once in each SOC and PT. Percentages will be based on the Safety Set. The SOCs will be listed in alphabetical order and PTs within each SOC will be listed by descending frequency. If needed, terms will also be ordered alphabetically.

Treatment-emergent SAEs, treatment-emergent non-SAEs, TEAEs leading to withdrawal of study drug, TEAEs leading to withdrawal from the study, TEAEs leading to death, and PTAEs will be summarized using the same approach.

#### 7.3.2.3. Adverse Events by System Organ Class

The number of TEAEs and the number and percentage of participants with events will be presented by SOC. Participants are counted once in each SOC only. Percentages will be based on the total number of treated participants in the treatment cohort.

#### 7.3.2.4. Adverse Events by Preferred Term

The number of TEAEs and the number and percentage of participants with events will be presented by PT. Participants are counted once in each PT only. Percentages will be based on the total number of treated participants in the treatment cohort.

#### 7.3.2.5. Adverse Events and Serious Adverse Events by System Organ Class, Preferred Term, and Relationship

The number of TEAEs and the number and percentage of participants with events will be presented by SOC and PT as described in Section 7.3.2.2 by relationship (related, not related). If a participant has more than one occurrence of an AE, the strongest relationship to study treatment will be used in the summary table. If relationship to study drug is missing, the AE will be assumed to be related. A similar analysis will be conducted for treatment-emergent SAEs.

The number of related TEAEs and the number and percentage of participants with related TEAEs will be summarized by SOC and PT, and separately by PT only. The same analyses will be produced for related treatment-emergent SAEs.

Lastly, the number of TEAEs by SOC, PT and relationship, without taking into account the highest relationship, will be analyzed. A similar analysis will be conducted for treatment-emergent SAEs.

#### 7.3.2.6. Adverse Events and Serious Adverse Events by System Organ Class, Preferred Term, and Toxicity

The number of TEAEs and the number and percentage of participants with events will be presented by SOC, PT and toxicity (ie, CTCAE grade). If a participant has more than one occurrence of an AE, the highest toxicity reported will be used. The number of TEAEs by SOC, PT and toxicity, without taking into account the highest toxicity, will also be analyzed.

Additionally, a summary of related TEAEs by SOC, PT and toxicity using the highest toxicity will be presented.

### 7.3.3. Other Safety

#### 7.3.3.1. Analyses for Laboratory Tests

Actual values and changes from treatment Baseline will be summarized descriptively for participants with available data for each laboratory parameter by cohort and overall. Missing laboratory data will not be imputed and only scheduled assessments will be included in by-visit summaries. All data, including that which is only collected at Screening, will be included in data listings. Laboratory measurements will be listed separately by participant, laboratory test, unit, and visit.

Laboratory parameter values will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE). Shift tables by treatment will be produced for these laboratory parameters. These tables will summarize the number of participants with each baseline grade relative to the reference ranges and changes to the worst highest grade assessed post dose during the study.

Summary results for shift will include the count and percentage of participants within each shift category by scheduled visit. 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 and an evaluation of drug-induced serious hepatotoxicity plot will be created.

#### 7.3.3.2. Vital Signs

Changes from Baseline in vital signs (blood pressure, heart rate, respiratory rate, and body temperature) at each visit will be summarized descriptively by cohort and overall. Missing vital signs data will not be imputed and only scheduled assessments will be summarized in tables; unscheduled assessments will be presented in data listings. A listing of vital signs will be presented by participant, cohort, vital sign, and visit.

#### 7.3.3.3. Physical Examination

Abnormal physical examination results will be tabulated by study visit and body system. Physical examination data (including height and weight) will also be listed by scheduled visit.

#### 7.3.3.4. Electrocardiogram

All ECG data will be fully listed and changes from Baseline in ECG data (heart rate, PR interval, RR interval, QRS duration, QT interval) will also be summarized descriptively by scheduled visit. Replicate samples will be averaged in these analyses, using the arithmetic mean.

All QT intervals will be corrected for heart rate according to Fridericia ($QTcF = QT/(RR^{1/3})$). At each time point, the number and percentage of participants falling into the following categories according to the International Conference on Harmonization (ICH) E14 Guidelines will be presented:

- QTc actual values: ≤450 ms, >450 to ≤480 ms, >480 to ≤500 ms, and >500 ms
- QTc increases from Baseline of >30 msec and >60 msec

Changes from Baseline in QTc will also be summarized. Further, ECG data will be classified by the Investigator as "normal," "abnormal, not clinically significant," "abnormal, clinically significant" or "indeterminate" at each timepoint assessed. Summary results will include the count and percentage of participants within each category at each visit.

## 7.4. Pharmacokinetic and Pharmacodynamic Analyses

For PK and PD endpoints, analyses will be performed using the PK and PD Analysis Sets respectively. The PK and PD concentration and parameters, as data permit, will be summarized by cohort.

### 7.4.1. Pharmacokinetic Concentration Analyses

Plasma concentrations of total molybdenum and PUF molybdenum (as surrogate measures of ALXN1840 PK) vs. time data will be presented in a data listing by participant. Plasma concentration data will be summarized separately by analyte, treatment (dose), day, and time point using the following descriptive statistics: number of participants, arithmetic mean, SD, coefficient of variation (CV), geometric mean (GM), GMCV, 95% confidence interval (CI), median, minimum, and maximum. When calculating the geometric mean, values of 0 will be discarded. For summary statistic calculations, plasma concentration values below the lower limit of quantification (LLOQ) will be set to the LLOQ for analyses using the Full Analysis Set and will be set to 0 for analyses using the PK Analysis Set.

Mean plasma concentration versus scheduled time profiles will be presented in figures on both linear and semilogarithmic scales. Individual plasma concentration versus actual time profiles will be presented similarly. The time to reach steady state will be graphically assessed by plotting mean plasma trough (pre-dose) concentration observed at the start of the dosing interval ($C_{trough}$) versus study day in both linear and semilogarithmic scales.

### 7.4.2. Pharmacodynamic Concentration Analyses

Individual ALXN1840 PD and biomarkers, assessed as plasma total copper (PD), PUF copper (PD), LBC (PD), dNCC (PD), Cp (biomarker) and CpC (biomarker) concentration-time data (including measured, absolute change from baseline and percent change from baseline) will be listed by participant.

The PD and biomarker concentration (measured, absolute change from Baseline and percent change from Baseline) data will be summarized separately by analyte, treatment (dose), day, and time point using the following descriptive statistics: number of participants, arithmetic mean, SD, CV, GM, GMCV, median, minimum, and maximum. When calculating the GM, values of 0 will be discarded. For PD and biomarker summary statistic calculations, concentrations below the LLOQ will be set to the LLOQ for analyses using the Full Analysis Set and will be set to 0 for analyses using the PD Analysis Set.

Mean PD and biomarker concentration (measured, absolute change from Baseline and percent change from Baseline) versus scheduled time profiles will be presented in figures on linear scales. Individual PD and biomarker concentration (measured, absolute change from Baseline and percent change from Baseline) versus actual time profiles will be presented similarly.

### 7.4.3. Pharmacokinetic Parameter Analyses

The following plasma PK parameters, as data permit, will be calculated for total molybdenum and PUF molybdenum (as surrogate measures of ALXN1840 PK) after dosing on Days 1, 25, 29 and 39 using noncompartmental methods with Phoenix® WinNonlin® (Certara USA Inc., Princeton, New Jersey) Version 8.0 or higher or SAS Version 9.4 or higher (SAS Institute Inc., Cary, North Carolina), as applicable. Calculations will be based on the individual actual sampling times relative to the actual ALXN1840 reference dosing times recorded during the study.

- Time delay between the time of dosing and time of appearance of molybdenum concentration ($T_{lag}$) in plasma
- Maximum observed concentration ($C_{max}$)
- Dose-normalized $C_{max}$ ($C_{max\_n}$); n = dose normalized
- Time to maximum concentration ($T_{max}$)
- Trough (pre-dose) concentration observed at the start of the dosing interval ($C_{trough}$)
- Time of last quantifiable concentration ($T_{last}$)
- Observed concentration at the end of the dosing interval ($C_{\tau}$, where $\tau$ = 24 h)
- Area under the plasma concentration versus time curve (AUC) from time 0 to the last quantifiable concentration ($AUC_t$)
- Dose-normalized $AUC_{t\_n}$ ($AUC_{t\_n}$); n = dose normalized
- AUC from time 0 to infinity ($AUC_{\infty}$) (Day 1 only)
- Dose-normalized $AUC_{\infty}$ ($AUC_{\infty\_n}$); n = dose normalized
- AUC over the dosing interval ($AUC_{tau}$)
- Accumulation ratio (AR) calculated as $C_{max}$, $C_{trough}$, and $AUC_{tau}$ after repeat dosing divided by those after initial dosing on Day 1:

  For 15 mg/day:

  - $C_{max,Day25}/C_{max,Day1}$
  - $C_{trough,Day26}/C_{trough,Day2}$
  - $AUC_{tau,Day25}/AUC_{tau,Day1}$

  For 30 mg/day:

  - $C_{max,Day39}/C_{max,Day29,adjusted}$
  - $C_{trough,Day40}/C_{trough,Day30,adjusted}$
  - $AUC_{tau,Day39}/AUC_{tau,Day29,adjusted}$

Note: total molybdenum and PUF molybdenum concentration-time profiles on Day 28 after the last 15 mg ALXN1840 dose will be extrapolated to Day 29 (0-24 hr) and subtracted from the

observed Day 29 concentration-time profiles after the first 30 mg ALXN1840 dose for the estimation of the $C_{max,Day29,adjusted}$, $C_{trough,Day30,adjusted}$, and $AUC_{tau,Day29,adjusted}$.

- Apparent terminal phase elimination rate constant ($\lambda_z$)
- Terminal elimination half-life ($t_{½}$)
- Apparent total body clearance (CL/F) of ALXN1840 from plasma
- Apparent volume of distribution ($V_d$/F)

For ALXN1840, equivalent molybdenum dose will be used to calculate total molybdenum CL/F and $V_d$/F values as described in Section 7.2.2. For ALXN1840 30 mg equivalent molybdenum dose is 6.66 mg.

Additional plasma PK parameters may be calculated if deemed appropriate.

For the PK analysis, values below the limit of quantification (BLQ) prior to the first measurable concentration will be set to zero whereas the rest of all other BLQ values will be treated as missing.

Pharmacokinetic parameters derived from plasma concentrations of total molybdenum and PUF molybdenum will be presented in data listings and summarized by analyte, treatment (dose), and day using the following descriptive statistics: number of participants, arithmetic mean, SD, arithmetic CV, GM, GMCV, 95% CI, median, minimum, and maximum. Geometric mean and geometric CV will be presented for $C_{max}$ and AUCs only. When calculating the geometric mean, values of 0 will be discarded.

### 7.4.4. Pharmacodynamic Parameter Analyses

The following plasma PD parameters, as data permit, will be calculated for plasma total copper, PUF copper, and LBC on Days 1, 25, 29, and 39 using noncompartmental methods with Phoenix® WinNonlin® (Certara USA Inc., Princeton, New Jersey) Version 8.0 or higher, or SAS Version 9.4 or higher (SAS Institute Inc., Cary, North Carolina), as applicable. Calculations will be based on the individual actual sampling times relative to the actual reference ALXN1840 dosing times recorded during the study.

- Maximum observed effect after dosing ($CE_{max}$)
- Time after dosing at which the maximum effect was observed ($TE_{max}$)
- Time after dosing at which the last quantifiable concentration was observed ($TE_{last}$)
- Trough (pre-dose) effect concentration observed at the start of the dosing interval ($CE_{trough}$)
- Area under the effect versus time curve (AUEC) from the start of dose administration to the last observed quantifiable concentration ($AUEC_t$)
- AUEC over the dosing interval ($AUEC_{tau}$)

Additional plasma PD parameters may be calculated if deemed appropriate.

For the PD measures plasma total copper, LBC, and dNCC parameter calculations, concentrations BLQ prior to the first measurable concentration will be set to zero; all other BLQ

values will be treated as missing. For PUF copper parameter calculations, all BLQ values will be set to zero.

Pharmacodynamic parameters derived from plasma concentrations of total copper, PUF copper, dNCC, and LBC will be presented in data listings and summarized by analyte, treatment (dose), and day the following descriptive statistics: number of participants, arithmetic mean, SD, arithmetic CV, GM, GMCV, median, minimum, and maximum.

### 7.4.5. Pharmacokinetic Statistical Analyses

Attainment of steady state will be evaluated using stepwise testing for linear trend.

Plasma total molybdenum and total copper $AUC_{tau}$ relationship will be assessed using linear least squares regression and $E_{max}$ model.

If data permit, dose-proportionality for ALXN1840 will be assessed graphically and by using a power model (Smith, 2000; Newlands, 2006; EMA, 2010). The following PK parameters obtained on Days 25 and 39 will be assessed for dose proportionality: $C_{max\_n}$ and $AUC_{t\_n}$. The log (PK parameters for plasma total and PUF molybdenum) will be included as a response variable and log (dose) will be included as a fixed effect in the power model:

$\log(\text{PK Parameter}) = \mu + \beta * \log(\text{Dose})$,

where $\beta = 1$ indicates perfect dose proportionality. Estimation of $\beta$, along with its 90% CI, will be provided.

Additional approaches to test dose proportionality, such as an ANOVA model, will be explored (Calvo, 2005).

# 8. REFERENCES

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# 9. APPENDICES

## 9.1. Sample Size, Power, and Randomization

The sample size will be approximately 10 participants which will allow a general characterization of copper balance in response to ALXN1840.

## 9.2. Technical Specifications for Derived Variables

### 9.2.1. Adverse Events

The analysis of AEs is described in detail in Section 7.3.2.

Treatment-emergent AEs are events with start dates and start times on or after the date and time of the first study drug dose. If the start date of an AE is partially or completely missing and the end (stop) date and time of the AE does not indicate that it occurred prior to first dose, then the determination of treatment-emergent status will be based on the following:

- If the start year is after the year of the first study drug dose, then the AE is treatment-emergent; 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 AE is treatment-emergent; else if
  - the start month is present and is the same or after the month of the first study drug dose, then the AE is treatment-emergent; else,
- If the start date is completely missing, then the AE is treatment-emergent.

All other AEs are considered PTAEs (ie, AEs with partial or complete start dates before first study drug dose).

### 9.2.2. Age and Dates

Age will be presented as the number of years between date of birth and the reference date. The following age in Table 2 may be computed, with reference date indicated.

**Table 2: Age and Reference Date**

| Age | Reference Date |
|---|---|
| • Age at Enrollment | • Date of informed consent |

The following formula should be followed for calculation of age if needed:

$$\text{Age (year)} = \text{FLOOR}(\text{reference date} - \text{date of birth})/365.25$$

where FLOOR( ) function returns the integer part of the result.

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 the day is observed but the month is missing, the date will be imputed as June 15. In instances when the imputed reference date is earlier than the birth date, the birth date will be used as the reference date.

### 9.2.3. Analysis Relative Day

Analysis relative day is the day relative to the first dosing day. It will be calculated as: analysis date – first dose date + 1 if analysis date is after the first dose date, or else as: first dose date – analysis date.

### 9.2.4. Baseline Value

The Baseline value is defined as the last non-missing value collected on or prior to first dose.

### 9.2.5. Body Mass Index

The BMI is derived as follows: weight (kg) / [height (cm) / 100]$^2$

### 9.2.6. Change from Baseline

Change from Baseline will be calculated as: post-Baseline assessment value – Baseline assessment value when both values are not missing.

Percent change from Baseline is calculated as (change from Baseline/Baseline result * 100).

If either the Baseline or the post-Baseline result is missing, the change from Baseline and/or percentage change from Baseline is set to missing. Additionally, if the Baseline is 0, the percentage change from Baseline will be missing.

### 9.2.7. Medications and Therapies

Medications/therapies with administration dates and times on or after the date and time of the first study drug dose are considered concomitant. 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 14 days prior to study enrollment up through the Screening Period and prior to the first dose.

### 9.2.8. 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. All visits will be assigned a target study day. Baseline will have a target study day of 1. For each assessment, the post-Baseline Period will be divided up using the scheduled visit's target day. The lower bound of each visit interval will be evaluated as the mid-point between the target day and the previous visit's target day in the following manner: study day interval lower bound = ceiling(target study day – ((target study day – last target study day)/2)). Table 3 shows an

example of visit windowing that will be used to summarize by-visit vital sign data. Other data will be summarized similarly in accordance to their SoA (Protocol Table 1).

**Table 3: Visit Windows used to Summarize by-Visit Vital Signs Data**

| Scheduled Visit | Target Study Day | Study Day Interval |
|---|---|---|
| Baseline | 1 | Last measurement on or prior to first dose of study drug |
| Day 9 | 9 | 2 to 15 |
| Day 23 | 23 | 16 to 31 |
| Day 40 | 40 | 32+ |

If more than one value is mapped to the same scheduled visit, the closer of those values will be considered for summarization. If multiple records exist with the same distance from the target study day, the last occurrence will be used. 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.

The visit displayed on data listings will be reflective of the scheduled visit label as reported on the CRF. 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.

## 9.3. Additional Details on Statistical Methods

Not applicable.