A Phase 1, Randomized, 2-Period, 2-Sequence, Crossover with Parallel-group Extension, Open-label Study to Compare the Relative Bioavailability of 2 Oral Formulations of ALXN1840 in Healthy Adult Participants

Unique Protocol ID:	ALXN1840-HV-109
NCT Number	NCT04610580
Date of SAP	11 March 2021

Statistical Analysis Plan for Interventional Studies

SAP Version Number: 1.0
SAP Date: 11-Mar-2021

Sponsor Name: Alexion Pharmaceuticals, Inc.

Protocol Number: ALXN1840-HV-109
Protocol Title: A Phase 1, randomized, 2-period, 2-sequence, crossover with parallel-group extension, open- label study to compare the relative bioavailability of 2 oral formulations of ALXN1840 in healthy adult participants

Protocol Version and Date: Protocol Amendment 2, 18-Dec-2020

Syneos Health Project Code: 7017347

Authors: PPD

Notice of Confidential and Proprietary Information:

I confirm that I have reviewed this document and agree with the content.

Approvals

Syneos Health Approval

PPD PPD	Signature	Date (DD-Mmm-YYYY)
PPD PPD	Signature	Date (DD-Mmm-YYYY)
PPD PPD	Signature	Date (DD-Mmm-YYYY)

Alexion Pharmaceuticals, Inc. Approval

PPD PPD Alexion Pharmaceuticals	Signature	Date (DD-Mmm-YYYY)
PPD Alexion Pharmaceuticals	Signature	Date (DD-Mmm-YYYY)

Table of Contents

1. Glossary of Abbreviations

Abbreviation	Description
$\%AUC_{extrap}$	AUC extrapolated from time t to infinity as a percentage of total AUC_{∞}
λ_z	Apparent terminal phase elimination rate constant
AE	Adverse Event
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area under the plasma concentration versus time curve
AUC_t	Area under the plasma concentration versus time curve from time 0 to the last quantifiable concentration
AUC_t_n	Dose-normalized AUC_t
AUC_{∞} / AUC_{inf}	Area under the plasma concentration versus time curve from zero to infinity
AUC_{∞}_n / AUC_{inf}_n	Dose-normalized AUC_{∞} / AUC_{nf}
BMI	Body Mass index
CI	Confidence Interval
CL/F	Aapparent oral clearance
C_{max}	Maximum observed concentration
C_{max}_n	Dose-normalized C_{max}
CRF	Case Report Form
CRU	Clinical Research Unit
CTCAE	Common Terminology Criteria for Adverse Events
Cu	Copper
CV	Coefficient of Variation
EC	Enteric-coated
ECG	Electrocardiogram
EMA	European Medicines Agency
EOS	End of Study
ET	Early Termination
FDA	Food and Drug Administration

F_{rel}	Relative bioavailability between test and reference treatments
FSH	Follicle-stimulating hormone
GMR	Geometric mean ratio
HbA1c	Glycated Hemoglobin
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IQR	Interquartile range
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
LBC	Labile bound copper
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
Mo	Molybdenum
N/A	Not Applicable
NCC	Non-ceruloplasmin-bound copper
$NCC_{corrected}$	NCC corrected for the amount of copper bound to the TPC
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PT	Preferred Term
PUF	Plasma ultrafiltrate
Q1	First quartile, 25th percentile of the data
Q3	Third quartile, 75th percentile of the data
QC	Quality Control
QTcF	QT interval corrected for heart rate using Fridericia's formula
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation

SE	Standard Error
SI	Standard International System of Units
SOC	System Organ Class
SOP	Standard Operating Procedure
$t_{½}$	Terminal elimination half-life
TEAE	Treatment-Emergent Adverse Event
TESAE	Treatment-Emergent Serious Adverse Event
TFL	Table, Figure and Listing
T_{lag}	time delay between the time of dosing and time of appearance of molybdenum concentration
t_{max}	time to maximum concentration
ULN	Upper limit of normal
V_d/F	Apparent volume of distribution
WD	Wilson disease
WHO	World Health Organization

2. Purpose

The purpose of this statistical analysis plan (SAP) is to ensure that the data listings, summary tables and figures which will be produced, and the statistical methodologies which will be used, are complete and appropriate to allow valid conclusions regarding the study objectives.

2.1. Responsibilities

Syneos Health will perform the statistical analyses and is responsible for the production and quality control of all tables, figures and listings

2.2. Timings of Analyses

The primary analysis of safety and pharmacokinetics (PK)/pharmacodynamics (PD) is planned after all participants complete the final study visit or terminate early from the study.

An interim analysis is planned after completion of the Two-way Crossover periods of the study to enable reporting of results that will inform the decision on safe and appropriate dosing with the 1.25 mg enteric-coated (EC) mini-tablets in the planned Study ALXN1840-WD-302 in pediatric patients with WD who will be required to be treated with the starting dose of 2.5 mg ALXN1840.

3. Study Objectives

Objective	Endpoints/Estimands
Primary	
To assess the relative bioavailability of equal doses of ALXN1840 administered as 1.25 mg EC mini-tablets versus a single 15 mg EC tablet	PK parameters for plasma total Mo and PUF Mo (C_{max}, AUC_t, and AUC_∞)
Secondary	
To assess dose-proportionality between 2.5 mg (2 × 1.25 mg), 5 mg (4 × 1.25 mg), 10 mg (8 × 1.25 mg), 15 mg (12 × 1.25 mg), and 30 mg (24 × 1.25 mg) EC mini-tablet doses	Dose-normalized PK parameters for plasma total Mo and PUF Mo (C_{max_n}, AUC_{t_n}, and AUC_{∞_n})
Safety	
To assess the overall safety and tolerability of ALXN1840, administered as 1.25 mg EC mini-tablets and as a single 15 mg EC tablet	Incidence of TEAEs and TESAEs, physical examination, vital signs measurements, clinical laboratory, and 12-lead ECG results
Exploratory	
To explore relationships between total Mo and PUF Mo clearance and body size — body weight (kg) and BMI (kg/m^2)	CL/F, body weight, and BMI
To explore PD of ALXN1840 either as a single 15 mg EC tablet or EC mini-tablets of 1.25 mg at different total dose strengths	Absolute and percent changes from pre-dose baseline of plasma Cu concentrations: total Cu and PUF Cu

Abbreviations: AUC_∞ = area under the plasma concentration versus time curve from time 0 to infinity; AUC_t = area under the plasma concentration versus time curve from time 0 to the last quantifiable concentration; BMI = body mass index; CL/F = apparent oral clearance; C_{max} = maximum observed concentration; Cu = copper; EC = enteric-coated; ECG = electrocardiogram; Mo = molybdenum; n = dose-normalized; PD = pharmacodynamic(s); PK = pharmacokinetic(s); PUF = plasma ultrafiltrate; TEAE = treatment-emergent adverse events; TESAE = treatment-emergent serious adverse event.

4. Study Details/Design

4.1. Brief Description

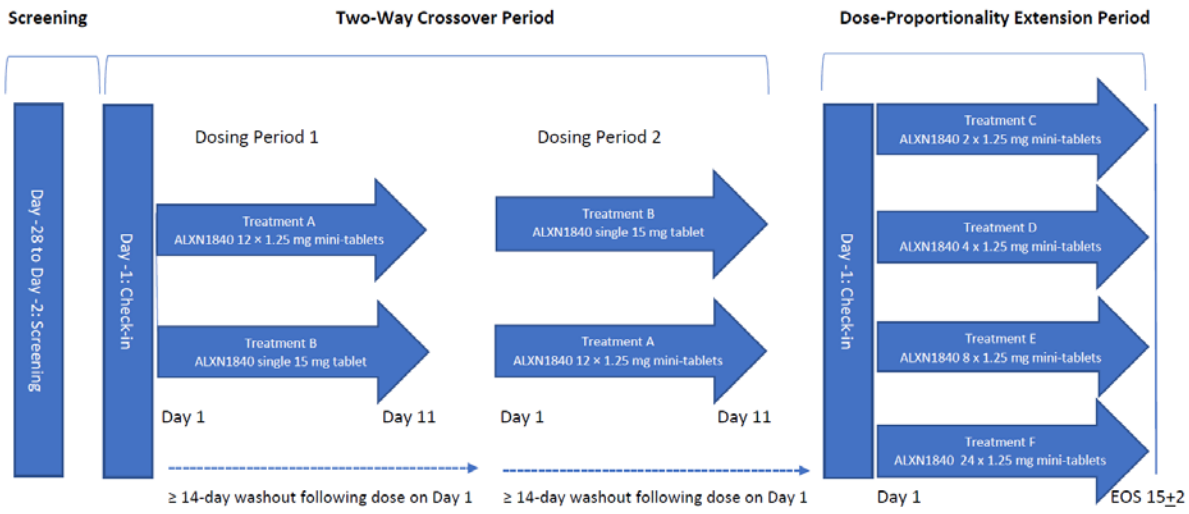
This is a Phase 1 study to compare the relative bioavailability of 2 formulations of ALXN1840 in healthy participants. Equal doses of the 1.25 mg EC mini-tablet will be compared with a single 15 mg EC tablet (12 × 1.25 mg and 1 × 15 mg, respectively). The study will be conducted in 2 periods. The study will also assess dose-proportionality between 2.5 mg (2 × 1.25 mg), 5 mg (4 × 1.25 mg), 10 mg (8 × 1.25 mg), 15 mg (12 × 1.25 mg), and 30 mg (24 × 1.25 mg) EC minitablet doses in the Dose-Proportionality Extension Period.

The study has a Screening Period (Days -28 to -2), the Two-way Crossover Periods, consisting of 2 dosing periods (Day 1 to Day 11 each), and a Dose-Proportionality Extension Period of 14 days. After completing the Screening Period, enrolled participants will be admitted to the clinical research unit (CRU) on Day -1 for dosing on Day 1 in Dosing Period 1. If discharged after Dosing Period 1, participants will be readmitted to the CRU for Dosing Period 2 following a minimum washout of 14 days after the previous dose, and again for the Dose-Proportionality Extension Period after a minimum washout of 14 days.

This study will be conducted with approximately 48 healthy participants enrolled to allow for a minimum of 40 participants to complete the Dose-Proportionality Extension Period of the study.

The study design is presented in Figure 1 .

Figure 1: Study Design Schematic



4.1.1. Two-way Crossover Periods

The Two-way Crossover Period is a randomized, open-label, two-way (2-period, 2-sequence) crossover design to assess the relative bioavailability of 12 × 1.25 mg EC mini-tablets compared with the 15 mg EC tablet currently used in clinical studies. Participants will be randomized to one of the 2 treatments

sequences. Randomized treatment assignment will be based on Baseline BMI. Two strata for Baseline BMI (< 25, 25 to < 32 kg/m^2) will be used:

- Treatment A: ALXN1840 12 × 1.25 mg EC mini-tablets
- Treatment B: ALXN1840 single 15 mg EC tablet, currently being tested in the Phase 3 Study WTX101-301)

Blood samples for PK analysis of total and PUF molybdenum (as surrogate measures of ALXN1840 PK) and PD/biomarkers will be collected in each dosing period on Day 1 at pre-dose, and postdose at 1, 2, 3, 4, 5, 6, 8, 12 and 24 hours (Day 2) and then at 24 hour intervals on Days 3, 4, 5, 6, 7, 8, 9, 10, and 11.

The 336-hour sample for Dosing Period 1 will be collected predose in Dosing Period 2. Participants may be discharged on Day 11 of each dosing period after completion of all procedures and review of all safety data. The end of Dosing Period 2 will occur on Day 15 ± 2 of Dosing Period 2, with the collection of the 336-hour PK sample for Dosing Period 2.

4.1.2. Dose-Proportionality Extension Period

The Dose-Proportionality Extension Period is a re-randomized, open-label, parallel group design to assess the dose-proportionality between EC mini-tablet doses.

The Dose-Proportionality Extension Period will be conducted following completion of the Two-way Crossover Periods of the study and after an at least 14-day washout period. Participants will be re-randomized as follows:

Treatment C (N=10-12): ALXN1840 2.5 mg (2 × 1.25 mg EC mini-tablets)

Treatment D (N=10-12): ALXN1840 5 mg (4 × 1.25 mg EC mini-tablets)

Treatment E (N=10-12): ALXN1840 10 mg (8 × 1.25 mg EC mini-tablets)

Treatment F (N=10-12): ALXN1840 30 mg (24 × 1.25 mg EC mini-tablets)

The 15 mg dose will not be repeated during the Dose-Proportionality Extension Period. For the purpose of evaluating dose-proportionality of the EC mini-tablet, data obtained from Treatment A of the Two-way Crossover Periods (12 × 1.25 mg EC tablets) will be included to represent a dose of 15 mg.

4.2. Participant Selection

Full eligibility criteria will be assessed only for Dosing Period 1 (ie, on Day -1). In- and exclusion criteria are described in detail in Protocol Sections 5.1 and 5.2.

4.3. Determination of Sample Size

A sample size of 48 male or female participants (24 participants in each sequence) has been selected based on practicality and convention for this type of study. Considering potential for drop-out of the study (approximately 15%), a total of 48 participants will be enrolled to ensure a minimum of 40 participants complete the study.

4.4. Treatment Assignment and Blinding

- This is an open-label, 2-period, 2-sequence study where, in order to minimize selection bias in

treatment assignment, each participant will be randomized to one of 2 treatment sequences (Treatment A and B) in the Two-way Crossover Periods. The randomization will be stratified by Baseline BMI. Two strata for Baseline BMI (< 25, 25 to < 32 kg/m^2) will be used.

- Eligible participants who meet all inclusion and no exclusion criteria included in the study will be assigned unique study participant numbers during randomization to one of 2 treatment sequences (Treatments A and B). Study participant numbers will not be reallocated once assigned.

- Participants will be re-randomized in the Dose-Proportionality Extension Period to one of 4 treatment sequences (Treatments C, D, E, and F). The re-randomized treatment assignment will be based on Baseline BMI as for the Two-way Crossover Periods, with 2 strata for Baseline BMI (< 25, 25 to < 32) to be used. Block randomization will be used to equally randomly assign participants to each treatment.

4.5. Administration of Study Medication

ALXN1840 will be administered with 240 mL (8 fluid ounces) of water after an overnight fast of at least 10 hours, as per FDA guidance. Participants can take approximately 5 minutes to drink the water. Participants will remain fasted for a minimum of 4 hours following each dose administration. Additional water is permitted ad libitum except for the period 1 hour before to 1 hour after administration of ALXN1840.

Details of ALXN1840 administered in the study are provided in Table 1:

Table 1: Details of Study Intervention Administered

ARM Name	**Treatments A, C, D, E, F**	**Treatment B**
Study Intervention Name	ALXN1840	ALXN1840
Type	Drug	Drug
Dose Formulation	White, round, EC mini-tablet, diameter 3 mm	White, round, EC tablet, diameter 5 mm
Unit Dose Strengths	1.25 mg EC mini-tablet supplied as 4 × 1.25 mg EC mini-tablets within a size 0 capsule. Capsules will be opened to access the individual mini-tablets (details will be provided in the Study Manual).	15 mg
Dosage Levels	Treatment A: 12 × 1.25 mg EC mini-tablets (15 mg) Treatment C: 2 × 1.25 mg EC mini-tablets (2.5 mg) Treatment D: 4 × 1.25 mg EC mini-tablets (5 mg) Treatment E: 8 × 1.25 mg EC mini-tablets (10 mg) Treatment F: 24 × 1.25 mg EC mini-tablets (30 mg)	15 mg
Route of Administration	Oral	Oral
Use	Test	Reference
Packaging and Labeling	ALXN1840 will be provided in bottles. Each bottle will be labeled as required per country requirements.	ALXN1840 will be provided in treatment kits. Each kit will be labeled as required per country requirements.

4.6. Study Procedures and Flowchart

Schedule of activities is in Protocol Section 1.3.

5. Endpoints

5.1. Primary PK Endpoint

PK parameters for plasma total molybdenum and PUF molybdenum (C_{max}, AUC_t, and AUC_∞)

5.2. Secondary PK Endpoints

Dose-normalized PK parameters for plasma total molybdenum and PUF molybdenum (C_{max_n}, AUC_{t_n}, and AUC_{∞_n})

5.3. Safety Endpoints

Incidence of TEAEs and TESAEs, physical examination, vital signs measurements, clinical laboratory, and 12-lead ECG results

5.4. Exploratory Endpoints

- CL/F, body weight, and BMI
- Absolute and percent changes from pre-dose baseline of plasma copper concentrations: total copper and PUF copper

6. Analysis Sets

6.1. Enrolled Set

The Enrolled Set will include all participants who agree to participate in the study following completion of the informed consent process and who satisfy the inclusion/exclusion criteria and are randomized. Unless specified otherwise, this set will be used for participant listings and summaries of participant disposition.

6.2. Safety Set

The Safety Set (SS) will include all participants who receive at least 1 dose of ALXN1840. Participants will be analyzed according to treatment received. The SS will be used for all analyses of safety endpoints.

6.3. Pharmacokinetic/Pharmacodynamic Set

6.3.1. Pharmacokinetic/Pharmacodynamic Set - Two-way Crossover Periods

The Pharmacokinetic/Pharmacodynamic Set for the Two-way Crossover Periods (PKDS-CO) will include all participants who receive at least 1 dose of ALXN1840 in the Two-way Crossover Periods and have evaluable PK data for total and/or PUF molybdenum (as surrogate measures of ALXN1840 PK) in plasma. Plasma concentrations will be summarized for all participants in the PKDS-CO. The exclusions from summary statistics for total and/or PUF molybdenum plasma concentrations and/or PK parameters will be defined based on review of protocol deviations (if any) prior to database lock by PK scientist and study statistician at the discretion of the sponsor. Pharmacokinetic and pharmacodynamic analyses will be performed using the PKDS-CO.

6.3.2. Pharmacokinetic Set/Pharmacodynamic - Dose-Proportionality Extension Period

The Pharmacokinetic/Pharmacodynamic Set for the Dose-Proportionality Extension Period (PKDS-E) will include all participants who receive at least 1 dose of ALXN1840 in the Dose-Proportionality Extension Period and have evaluable PK data for total and/or PUF molybdenum (as surrogate measures of ALXN1840 PK) in plasma. Plasma concentrations will be summarized for all participants in the PKDS-E. The exclusions from summary statistics for total and/or PUF molybdenum plasma concentrations and/or PK parameters will be defined based on review of protocol deviations (if any) prior to database lock by PK scientist and study statistician at the discretion of the sponsor. Pharmacokinetic and pharmacodynamic analyses will be performed using the PKDS-E.

6.4. Full Analysis Set

The Full Analysis Set (FAS) will include all participants who receive at least 1 dose of ALXN1840 and who have sufficient plasma samples to have evaluable PD data for total copper or PUF copper. Participants will be analyzed according to treatment received. The FAS will be used for additional analyses of PD endpoints.

7. General Aspects for Statistical Analysis

7.1. General Methods

- In general, descriptive statistics for continuous variables will include number of non-missing values, arithmetic mean, SD, median,interquartile range (IQR), Q1, Q3, minimum, and maximum. Categorical variables will be summarized using percentages and frequency counts. Descriptive statistics and percentages and frequency counts will be summarized by treatment, and period, where appropriate.
- For the summary statistics of all continuous variables unless otherwise specified, minimum and maximum will be presented to the same number of decimal places as the raw data, mean, median, Q1, Q3, and IQR will be presented to one more decimal places than the raw data, and SD will be presented to two more decimal places than the raw data.
- All statistical analyses will be conducted using SAS® for Windows® Version 9.4 or higher.
- All relevant participant data will be included in listings. All participants entered into the database will be included in participant data listings.

In general, tables will be generated separately for the Two-way Crossover Periods and the Dose-Proportionality Extension Period.The tables for the Two-way Crossover Periods will present columns of treatments A, B and the tables for the Dose-Proportionality Extension Period will present columns of treatments C, D, E, F and a total column (see table shells for further details).

7.2. Key Definitions

End of Study (EOS)
The EOS for each participant is defined as completion of the EOS Visit on Day 15 ± 2 days of the Dose-Proportionality Extension Period.

Nominal Time
Nominal time is the scheduled measurement time relative to time 0. Time 0 is the time of study drug dosing on Day 1.

Baseline Value
Baseline value will be defined as the last non-mising value recorded prior to intake of study treatment in each dosing period.

Change from Baseline (CFB)
CFB = Post-baseline value – Value at baseline

7.3. Missing Data

For participants who are withdrawn from the study prior to the end of the study, all data collected up to the point of discontinuation will be used for analysis. There will be no imputation for missing data, unless otherwise specified.

7.4. Visit Windows

If there are multiple planned assessments for any study procedure at a given time point the latest non-missing value will be used for summarization. Unscheduled assessments will be listed and will be used to flag baseline visit if this is the last non-missing assessment before the first dose of study drug, but unscheduled assessments will not be included in the summarization.

Blood samples for assessing plasma PK/PD should be collected within ± 10% in minutes, or 30 minutes relative to the scheduled time, whichever is less.

7.5. Pooling of Centers

Not Applicable.

7.6. Subgroups

Not Applicable.

8. Participant Disposition, Demographic, Other Baseline Characteristics and Medication

8.1. Participant Disposition and Withdrawals

Listings of treatment assignments, including the participant's identification, date of randomization, and assignment to treatment sequence, will be presented.

The following frequencies (number and percent) will be displayed for all participants in the Enrolled Set: participants enrolled, participants randomized, screen failures, COVID-19 related reasons for screen failure.

The analysis sets will be summarized with counts and percentages by treatment and overall. This table will include the following: number of participants in Enrolled Set, participants in Safety Set, reasons for exclusion from the Safety Set, participants in PK/PD sets, reasons for exclusion from the PK/PD sets, number of participants Full Analysis Set, reasons for exclusion from the Full Analysis Set.

The following frequencies (number and percent) will be displayed for all participants in the Enrolled Set: randomized participants, participants in the Safety Set, participants in the PK/PD Sets and participants in the Full Analysis Set, participants who completed each treatment period, participants who completed the study (including follow-up), participants who discontinued early, and participants who discontinued study treatment early also presented by reason for early discontinuation. The denominators will be the number of enrolled participants. This table will be repeated for all analysis sets.

Completion/discontinuation status, inclusion/exclusion criteria definitions and Inclusion/exclusion criteria violations will be listed by participant.

A listing of participants excluded from the analysis populations will also be generated.

8.2. Protocol Deviations

Protocol deviation management at Syneos Health is detailed in Protocol Deviation and Non-compliance Management (3101.W02). For details on the process for defining analysis datasets refer to (Blind) Data Review and Definition of Analysis Sets SOP (3911).

All protocol deviations recorded in Clinical Trial Management System (CTMS) will be listed. The Protocol deviation criteria are graded as not important or important. Protocol deviations and Protocol deviations related to COVID-19 will be summarized by the number and percentage of participants for the Safety Set.

8.3. Demographic and Baseline Characteristics

Demographic and baseline characteristics, including age, sex, race, ethnicity, child-bearing potential, height, body weight, and BMI will be summarized using standard descriptive statistics. No formal statistical comparisons between populations will be performed. Demographics and informed consent data will be listed for all participants in the Enrolled Set.

Height (in cm) = height (in inches) * 2.54

Weight (in kg) = weight (in lbs) * 0.4536

8.4. Medical History and Concomitant Diseases

Medical history will be coded using MedDRA 23.1 and summarized for the Safety Set presenting the number and percentages of participants within each preferred term (PT) grouped by the system organ class (SOC). A participant with multiple occurrences of an event in a PT is counted only once. Medical history will be listed by participant.

8.5. Medication

The WHO Drug, March 2018, B3 will be used to classify prior and concomitant medications by therapeutic class and drug name.

Prior medication is defined as any medication taken before the date of the first dose of study treatment. Concomitant medication is defined as any medication taken on or after the date of the first dose of study treatment.

Treatment/period attribution will be determined by comparing the start date and time of the concomitant medication with the actual date and time of dosing. The most recently administered treatment prior to the date and time of onset will be considered as the treatment/period at onset. If a concomitant medication is ongoing over more than one treatment/period it will be attributed to all treatments/periods.

The use of prior and concomitant medications will be summarized by the number and percentage of participants for the Safety Set. If a participant takes a specific medication multiple times or takes multiple medications within a specific therapeutic class, that participant would be counted only once for the coded drug name or therapeutic class.

8.6. Non-Pharmacologic Therapies or Procedures

The WHO Drug, March 2018, B3 will be used to classify non-pharmacologic therapies by therapeutic class. Prior non-pharmacologic therapy is defined as any non-pharmacologic therapy taken before the date of the first dose of study treatment. Concomitant non-pharmacologic therapy is defined as any non-pharmacologic therapy taken on or after the date of the first dose of study treatment.

Treatment/period attribution will be determined by comparing the start date and time of the non-pharmacologic therapy with the actual date and time of dosing. The most recently administered treatment prior to the date and time of onset will be considered as the treatment/period at onset. If a non-pharmacologic therapy is ongoing over more than one treatment/period it will be attributed to all treatments/periods.

Non-pharmacologic procedure data will be listed but not summarized.

8.7. Extent of Exposure

Exposure data will be listed but not summarized.

8.8. Treatment Compliance

Not Applicable.

9. Pharmacokinetics

Pharmacokinetic analyses will be performed using the PKDS-CO and PKDS-E.

PK parameters will be calculated for total and PUF molybdenum (as surrogate measures of ALXN1840 PK) using noncompartmental methods with Phoenix® WinNonlin® (Certara USA Inc., Princeton, New Jersey) Version 8.0 or higher. Calculations of individual PK parameters will be based on measured total molybdenum and, when feasible, PUF molybdenum concentrations and the actual sampling times elapsed from the actual reference dosing time data recorded during the study.

9.1. PK Sampling Schedule

Two-way cross-over treatment periods

Blood samples for PK analysis of total and PUF Mo (as surrogate measures of ALXN1840 PK) and pharmacodynamic (PD)/biomarkers will be collected in each dosing period on Day 1 at pre-dose, and postdose at 1, 2, 3, 4, 5, 6, 8, 12 and 24 hours (Day 2) and then at 24 hour intervals on Days 3, 4, 5, 6, 7, 8, 9, 10, and 11.

The 336-hour sample for Dosing Period 1 will be collected predose in Dosing Period 2. Participants may be discharged on Day 11 of each dosing period after completion of all procedures and review of all safety data. The end of Dosing Period 2 will occur on Day 15 ± 2 of Dosing Period 2, with the collection of the 336-hour PK sample for Dosing Period 2.

Dose-proportionality extension period

The Dose-Proportionality Extension Period will be conducted following completion of the Two-way Crossover Periods of the study and after an at least 14-day washout period.

A similar schedule for PK samples collection will be followed in the dose proportionality extension period as two-way cross-over treatment periods. Further details are given in Section 4.6.

9.2. Plasma PK Endpoint

Following PK parameters will be derived from the plasma concentrations of total and PUF molybdenum when feasible and supported by the available data.

- Time delay between the time of dosing and time of appearance of drug concentration in plasma (Tlag; for plasma total molybdenum with ALXN1840 administration)
- Maximum observed concentration in plasma (C_{max})
- Molybdenum dose-normalized C_{max} (C_{max}_n); n = dose normalized
- Time to C_{max} (T_{max})
- Area under the plasma concentration (AUC) versus time curve from time 0 to the last quantifiable concentration (AUC_t)
- Molybdenum dose-normalized AUC_t (AUC_t_n)
- AUC versus time curve from time 0 to infinity (AUC_∞)

- Molybdenum dose-normalized AUC_{∞} (AUC_{∞}_n)
- AUC extrapolated from time t to infinity as a percentage of total AUC_{∞} ($\%AUC_{extrap}$)
- Apparent terminal-phase elimination rate constant (λ_z)
- Terminal elimination half-life ($t_{½}$)
- Apparent oral clearance (CL/F)
- Apparent volume of distribution (V_d/F)
- Relative bioavailability (F_{rel}) between test and reference treatments

Additional plasma PK parameters may be calculated if deemed appropriate.

9.3. Presentation of Concentration Data

9.3.1. Handling of Missing Data

Missing concentration data for all participants who are administered scheduled study treatments will be considered as non-informative missing and will not be imputed. No concentration estimates will be provided for missing sample values.

For the derivation of AUC and for the individual plasma concentration versus time curves, the following rules will apply:

- Concentration values below the assay's lower limit of quantification (BLQ) in pre-dose Day 1 samples will be treated as zero;
- The sampling time of pre-dose samples relative to dosing will also be treated as zero;
- BLQ's between two quantifiable samples will be set to missing;
- The first BLQ after the last measurable sample will be set to missing; any subsequent BLQ's will be set to zero;

For plasma concentration summary the following rules will apply:

- All BLQ values will be set to zero;
- No further imputation will be applied to any missing values.

9.3.2. Listing and Presentation of Individual PK Data

The actual and nominal sampling times of PK blood sample collection will be listed for each participant and will include the deviation in time from the protocol scheduled time (i.e., nominal time), if applicable.

All measured concentrations will be presented in original precisions (i.e., significant figures) and units as reported by Bioanalytical lab – eg ng/mL.

Plasma concentrations of total and PUF molybdenum and time deviation data will be presented in a data listing by participant.

9.3.3. Summary of PK Concentrations by Matrix

Plasma concentration data will be summarized separately by time point for each treatment using the following descriptive statistics: number of participants, arithmetic mean, SD, CV, geometric mean, geometric CV, median, minimum, and maximum.

Mean plasma concentration versus scheduled time profiles will be presented in figures on both linear and semilogarithmic scales. Individual plasma concentration versus nominal time profiles will be presented similarly.

9.4. Derivation of Pharmacokinetic Parameters

- The apparent C_{max} and the corresponding t_{max} along with T_{lag} will be read directly from the concentration-time plot (observed data).
- AUC_t will be calculated using the log-linear trapezoidal interpolation rule for extravascular model.
- The terminal elimination rate constant (λ_z) will be determined by log linear regression obtained on at least the 3 last quantifiable concentrations and will not include C_{max}; $t_{1/2}$ is calculated by the program as $\ln 2/\lambda_z$;
- AUC_∞ will be calculated by the program as: $AUC_\infty = AUC_t + AUC_{extrap}$ where last is the sampling time point of the last measurable concentration (t_{last}). AUC_{extrap} will be calculated by the program as: C_{last}/λ_z, where C_{last} is the observed concentration at time t_{last} and λ_z is the elimination rate constant during the apparent terminal elimination phase. AUC_∞ will only be presented for participants with a reliable λ_z;
- CL will be calculated by program as (molybdenum dose amount/AUC_∞);
- V_z will be calculated by the program as (molybdenum dose amount/AUC_∞)/λ_z;
- Dose normalized PK parameters will be calculated using the equivalent molybdenum dose and not the ALXN1840 dose. .

The following PK acceptance criteria will be applied to assess the reliability of elimination parameters:

- Number of points to calculate λ_z is greater than or equal to 3 excluding C_{max} point;
- The adjusted square of the correlation coefficient or coefficient of determination (Rsquare adjusted) for the goodness of fit of the regression line through the data points must be ≥0.90*;*
- AUC_{extrap} ≤20%.

Unreliable PK parameters will be listed but flagged and excluded from summary.

9.4.1. PK Parameters Summarization

Pharmacokinetic parameters derived from plasma concentrations of total and PUF molybdenum will be presented in data listings and summarized separately using the following descriptive statistics: number of participants, arithmetic mean, SD, arithmetic CV, geometric mean, geometric CV, median, minimum, and maximum.

9.5. Planned Statistical Models for PK Parameters and Concentrations

The primary analysis will be based on the PKDS-CO.

For the Period 1 and Period 2 comparisons, the PK parameters for total and PUF molybdenum (C_{max}, AUC_t, and AUC_∞) will be evaluated using an ANOVA statistical model with dosing period, treatment, and sequence as the fixed effects, and participant(sequence) as a random effect, using the natural logarithms of the data. Confidence intervals (CI, 90%) will be constructed for the least squares geometric mean ratio (GMR) for the 12 × 1.25 mg ALXN1840 mini-tablets versus a single 15 mg ALXN1840 tablet dose for all 3 PK parameters using the natural log-transformed data. The GMRs and the associated 90% confidence limits will be exponentiated back to the original scale.

The within-subject CV for the C_{max}, AUC_t, and AUC_∞ will be estimated using the mean squared error from the ANOVA.

In addition, the geometric means and the associated 95% CIs of C_{max}, AUC_t, and AUC_∞ will be reported for each treatment.

All secondary analyses will be based on the PKDS-E.

Dose-proportionality will be assessed across all EC mini-tablet treatments (2.5 mg, 5 mg, 10 mg, 15 mg, 30 mg ALXN1840) graphically and by using a power model (Smith, 2000; Newlands, 2006; EMA, 2010).

The following molybdenum dose-normalized PK parameters will be assessed for dose proportionality: C_{max_n}, AUC_{t_n} and AUC_{∞_n}. The log (PK parameters for plasma total and PUF molybdenum) will be included as a response variable and log (molybdenum dose) will be included as a fixed effect in the power model:

log(PK Parameter) = mu + beta*log(Molybdenum Dose)

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

9.6. Deviation from Analyses Planned in Protocol

Not applicable.

10. Exploratory Analysis

10.1.1. Endpoints

The endpoints are: Clearance of total molybdenum, clearance of PUF molybdenum, covariates such as body weight (kg), and BMI(kg/m^2).

10.1.2. Analysis of the Endpoint

Exploratory analyses as described in this section will be performed using the PKDS-CO and PKDS-E.

Box-whisker plots for two strata of BMI (< 25, 25 to < 32 kg/m2) (on X-axis) and corresponding molybdenum dose normalized clearance of total molybdenum and PUF molybdenum (on Y-axis) will be presented.

For body weight, a scatter plot with a Pearson correlation will be presented with body weight on X-axis and clearance of total molybdenum and PUF molybdenum on Y-axis to demonstrate if any correlation exists between the two. If needed, Pearson correlation analyses may be performed.

11. Pharmacodynamics

Samples for assessment of plasma total copper and PUF copper will be used for the PD analysis described in this SAP/section. Samples for the assessment of ceruloplasmin (Cp), ceruloplasmin-bound copper (CpC), non-ceruloplasmin-bound copper (NCC), and LBC will be retained and may be analyzed in the future.

Plasma samples of total copper and PUF copper will be collected on Day 1: Pre-dose, 1, 2, 3, 4, 5, 6, 8, 12 hours post-dose, Day 2, 3, 4 , 5 , 6 , 7 , 8 , 9, 10, 11 of each treatment period, details have been provided in Section 4.6.

11.1. PD Endpoint and Analysis

11.1.1. Endpoint

Absolute and change and percent change from pre-dose baseline of plasma copper concentrations: total copper and PUF copper

11.1.2. Analysis of the Endpoint

Pharmacodynamic analyses will be performed using the PK/PD Sets. For the plasma total copper and PUF copper concentration summaries BLQ values will be set to zero, but no further imputation will be applied to any missing values.For the plasma total copper and PUF copper concentrations, actual values, changes from baseline and percentages change from baseline will be summarized by time point for each treatment.

Mean plasma total copper and PUF copper concentration (absolute and change and percentage change from baseline) versus scheduled time profiles will be presented in figures.

Individual plasma total copper and PUF copper concentration (absolute and change and percentage change from baseline) versus scheduled time profiles will be presented similarly.

Plasma concentrations of total and PUF copper, absolute and change and percentage change from baseline will be presented in a data listing by participant.

Biostatistics summaries of total copper and PUF copper will be repeated for the FAS using the same methods as described above. In these analyses values BLOQ will be set to LLOQ.

12. Safety

The population used for safety analyses will be the Safety Set (SS).

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

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

12.1. Adverse Events / Adverse Drug Reactions

AEs for all participants in the Safety Set will be included in the AE summaries.

Adverse events will be summarized by system organ class (SOC) and preferred term (PT) for each treatment and overall, based on the MedDRA dictionary version 23.1.

If the relationship to study drug is missing for TEAEs, then the relationship will be counted as related to study product for the summary tables. Missing severity for TEAEs will be counted as Grade 3.

Treatment-emergent AEs are defined as AEs which commence on or after first study drug administration, or those that first occur before first study drug administration but worsen in frequency or severity after first study drug administration.

Treatment/period attribution will be determined by comparing the start date and time of the AE with the actual date and time of dosing. The most recently administered treatment prior to the date and time of onset will be considered as the treatment/period at onset. An AE will only be attributed to the treatment/period when it started, even if it is ongoing over more than one treatment/period.

If the start date of an AE is partially or completely missing, the date will be compared as far as possible with the date of the start of administration of study drug. The AE will be assumed to be treatment-emergent if it cannot be definitively shown that the AE did not occur or increase in severity during the treatment period (worst case approach). The following general rules will be used:

- If the start day is missing but the start month and year are complete, an AE will not be considered as being treatment-emergent only if the start month/year is before the month/year of the first study drug administration.
- If the start day and month are missing but the start year is complete, an AE will not be considered as being treatment-emergent only if the start year is before the year of the first Study Drug administration.
- If the start date is completely missing, an AE will be considered treatment-emergent unless the stop date is before the first study drug administration.

Time since first/last dose will be calculated as the difference between the event start date/time and the date/time of the first/last dose prior to start. Time since first/last dose will be expressed in days, hours and minutes. If time is missing, duration will be calculated as the difference between the start date and dosing date plus 1 and expressed in days. Time since first/last dose and duration will only be calculated when both dates are complete.

Duration will be calculated for AEs that resolve as the difference between the resolution date and onset date plus 1 and expressed in days.

The summary tables will include the number of participants and the number of events. Percentages will be based on the number of participants. For summaries by SOC and PT, a participant will be counted once at the SOC level and once at each PT within the SOC level.

For summaries by SOC, PT, and maximum severity, a participant will be counted once at the highest severity level for which the event occurred at the SOC level and the highest severity level for each unique PT within that SOC level. Therefore, participants may only contribute once to each PT and once to each SOC level.

The summaries presenting frequency of AEs by SOC and PT will be ordered by overall descending frequency of SOC and then, within a SOC, by overall descending frequency of PT.

The following tables will be provided:

- An overall summary of the number and percentage of participants reporting TEAEs, serious TEAEs (TESAEs), TEAEs resulting in death, TEAEs leading to withdrawal of study drug, TESAEs leading to withdrawal of study drug, TEAEs by relationship and TEAEs by toxicity grade;
- TEAEs overall by system organ class and preferred term;
- TEAEs by maximum toxicity grade, overall and by system organ class and preferred term;
- TEAEs by toxicity grade, overall and by system organ class and preferred term;
- TEAEs by maximum relationship to study medication, overall and by system organ class and preferred term;
- TEAEs by relationship to study medication, overall and by system organ class and preferred term;
- TEAEs by system organ class sorted by decreasing frequency;TEAEs by preferred term sorted by decreasing frequency;
- Related TEAEs by preferred term sorted by decreasing frequency;
- TESAEs, overall and by system organ class and preferred term;
- TESAEs by maximum relationship to study medication, overall and by system organ class and preferred term;
- TESAEs by relationship to study medication, overall and by system organ class and preferred term;
- Non-serious TEAEs overall and by system organ class and preferred term;
- TEAEs leading to withdrawal of study drug, overall and by system organ class and preferred term;
- TEAEs leading to death, overall and by system organ class and preferred term;

Only TEAEs will be included in the summary tables, however we will generate separate listings for treatment-emergent and non-treatment-emergent AEs. Additional listings will be provided for deaths, serious adverse events, AEs resulting in withdrawal from the study and Adverse Events Leading to Study Drug Withdrawal.

12.2. Laboratory Evaluations

Safety laboratory samples for hematology, chemistry, urinalysis and coagulation will be collected at Screening, Day 1, 2, 5 and 10 of each treatment period and EOS/ET.

Samples for pregnancy test will be collected at Screening, Day -1 of each treatment period and EOS/ET.

Samples for alcohol breath test and urine drug screen will be collected at Screening and Day -1 of each treatment period.

Samples for FSH, HIV antibody/hepatitis B and C screen and serum Ceruloplasmin and serum copper will be collected at Screening only.

The following parameters will be included:

Hematology: Platelets, White blood cell count with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), Mean cell hemoglobin, Neutrophils, Monocytes, Basophils, Hemoglobin, Mean corpuscular volume, Lymphocytes, Eosinophils.

Clinical chemistry: Blood urea nitrogen, Potassium, Creatinine, Creatine kinase, Sodium, Chloride, Glucose, HbA1cb (only at screening), Bicarbonate, AST, Gamma glutamyltransferase, ALT, Alkaline phosphatase, Urea, Magnesium, Total and direct bilirubin, Total protein, Albumin, Calcium, Phosphate.

Urinalysis: Bilirubin, Glucose, Leukocytes, Nitrite, Protein, Urobilinogen, Blood, Ketones, Microscopy, if required, pH, Specific gravity, Red blood cells.

Coagulation: Prothrombin time - International Normalized Ratio, Partial thromboplastin time.

Serology: Human immune deficiency virus (HIV)-1 and HIV-2 antibodies, hepatitis B surface antigen, and anti-hepatitis C virus with confirmation by hepatitis C virus RNA

Highly sensitive serum or urine human chorionic gonadotropin pregnancy test (as needed for women of childbearing potential), Follicle-stimulating hormone (postmenopausal females only)

Alcohol breath and **urine drug screen**: amphetamines, barbiturates, benzodiazepines, cocaine, opiates, phencyclidine, methamphetamine, 3,4-methylenedioxy-methamphetamine, methadone, and tetrahydrocannabinol [cannabinoids].

Other tests:Serum Ceruloplasmin, serum copper

Laboratory parameter values will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE).

All summaries will be based on results in SI (standard international system of units) units and will be output in the order listed above.

Toxicity grading following the NCI CTCAE grades version 5.0 will also be derived for laboratory variables where applicable (see list below). Note, where gradings require investigator/clinical input, this will not be considered and only the numeric criteria will be used for the assignments. If this results in the criteria for more than one grade being met, the highest (worst) CTCAE grade will be assigned. For creatinine only, the criteria based on the upper limit of normal (ULN) will be used (changes compared to the baseline value will not be used), eg, CTCAE defines Grade 1 as > ULN - 1.5 x ULN or > 1 - 1.5 x baseline. Only the ULN – 1.5 x ULN criterion will be used (and not the baseline criterion).

The following parameters are CTCAE gradable:

• Hematology:Absolute neutrophil count (neutrophil) , total leukocytes (Leukocytes) , hemoglobin (only do

the grading for Anemia, no grading for Hemoglobin increased), platelet count , Lymphocyte

• Coagulation:International normalized ratio

• Chemistry: Alanine aminotransferase albumin, alkaline phosphatase, aspartate aminotransferase, total bilirubin, creatinine, gamma-glutamyl transferase , glucose (only do the grading the Hypoglycemia, no grading for Hyperglycemia), creatine kinase.

Actual values and changes from baseline (screening) in hematology, coagulation, clinical chemistry, urinalysis will be summarized by treatment.

Shift tables for hematology, clinical chemistry and coagulation from baseline CTCAE toxicity for each visit and to the maximum grade on treatment for each parameter will be provided. Participants with both a non-missing baseline and at least one non-missing post-baseline grade will be included in the shift tables. Unscheduled data will be included in the worst case across all scheduled and unscheduled visits after the first dose of study treatment. This means that if there are CTCAE grades derived from both unscheduled and scheduled visits data per test per participant then the highest grade will be summarized.

For non-CTCAE gradable tests, a shift table will be provided showing shifts relative to the normal ranges. This summary of normal range category changes illustrates the number and percentage of participants who fall into specified categories (Decrease to Low, Change to Normal or No Change, Increase to High) by comparing the baseline normal range category to the planned time normal range category and the worst-case normal range category. The worst-case post-baseline row will be used to summarize the participants' overall worst-case normal range category change. The determination of the worst-case takes into account both planned and unscheduled assessments. Only laboratory tests which cannot be graded per CTCAE v. 5.0 specified criteria will be included.

Participants with missing baseline value are to be assumed to have normal baseline value. Worst-case can be either High or Low. If a participant has a Decrease to Low and an Increase to High during the same time interval, then the participant is counted in both the 'Decrease to Low' and 'Increase to High' categories. If a participant was high at baseline and decreases to Low during the time interval, then the participant is counted in the 'Decrease to Low' category. Likewise, if a participant was Low at baseline and increases to High during the time interval, then the participant is counted in the 'Increase to High' category. Participants are only counted in the 'Change to Normal or No Change' category if they are:

- Normal at baseline and have no normal range High and no normal range Low values during the time interval
- High at baseline and do not change to Low during the time interval
- Low at baseline and do not change to High during the time interval 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.

All events of ALT ≥ 3 × upper limit of normal (ULN) and bilirubin ≥ 2 ×ULN (> 35% direct bilirubin) or ALT ≥ 3 × ULN and international normalized ratio (INR) > 1.5, if INR measured which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE. Liver function results will be presented in a

summary table and eDISH (evaluation of drug-induced serious hepatotoxicity) plots.

All laboratory results, as well as CTCAE grades will be included in data listings. Abnormal results for hematology, clinical chemistry, urinalysis and coagulation will be listed separately. Laboratory results for serology, pregnancy tests, FSH, urine drug screen, alcohol breath test, serum Ceruloplasmin and serum copper will be listed only.

12.3. Vital Signs

Systolic blood pressure (mmHg), diastolic blood pressure (mmHg), heart rate (bpm), respiratory rate (breaths per minute) and temperature (degree Celsius) will be measured at Screening, Day 1 (Pre-dose, 6 hours, 12 hours), Day 2, Day 5, Day 11 of each treatment period and EOS/ET.

If vital sign results are abnormal, 2 additional readings will be taken. The mean of the 3 replicates will be recorded in the case report form (CRF) and used for the analysis.

Actual values and changes from baseline in vital sign measurements will be summarized by treatment.

Note temperature will be reported in centigrade. Any conversions required will be as follows:

Temperature (in °C) = 5/9 (Temperature [in °F]-32).

All vital signs will be provided in data listings.

12.4. ECG

The ECG parameters include heart rate, PR, RR, QRS, QT, and corrected QTcF intervals.

12-lead ECGs in triplicate will be taken at Screening and on Day 1, 0.5 hours pre-dose and at 6 hours post-dose on Day 1 of the first dosing period and Day 1 of the Dose-Proportionality Extension Period. 12-lead ECGs should be measured after a minimum of 5 minutes in supine position. The average of the triplicate ECG readings at the time points collected will be calculated and used in the analyses.

Actual values and change from baseline of these average ECGs will be summarized by treatment.

All ECG parameters, including all single ECG measurements, but also the mean value and overall ECG evaluation will be provided in data listings.

12.5. Physical Examination

A full physical examination will be performed at Screening and on Day -1 of the 2 dosing periods. A symptom-driven physical examination of relevant body systems may be performed at other times, at the Investigator's discretion.

Physical examination data will be listed.

13. Interim Analyses

An interim analysis is planned after completion of the Two-way Crossover Periods of the study (Group 2 discharged after completing Period 2) and after the bioassay results for plasma total and PUF Mo and Cu results become available.

The interim analysis will capture all PK, PD, and safety data and provide comparative relative bioavailability data of the 2 treatments to decide on safe and appropriate dosing with the 1.25 mg EC mini-tablets in the planned Study ALXN1840-WD-302 in pediatric patients with WD.

14. Changes from Analysis Planned in Protocol

Not Applicable.

15. Reference List

- EMA. Committee for medicinal products for human use (CHMP): Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1). Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf. 2010.
- Newlands A. Statistics and Pharmacokinetics in Clinical Pharmacology Studies. SAS Conference Proceedings: Pharmaceutical Users Software Exchange; Dublin, Ireland, 2006.
- Smith BP, Vandenhende FR, DeSante KA, et al. Confidence interval criteria for assessment of dose proportionality. Pharm Res. 2000;17(10):1278-1283.

16. Quality Control

SAS programs are developed to produce output such as analysis data sets, summary tables, data listings, figures or statistical analyses. An overview of the development of programs is detailed in Syneos Health Developing Statistical Programs SOP (3907) .

Syneos Health Developing Statistical Programs SOP (3907), Conducting the Transfer of Biostatistical Deliverables SOP (3908) and the SAS Programming and Vaidation Plan describes the quality control procedures that are performed for all SAS programs and output. Quality control is defined here as the operational techniques and activities undertaken to verify that the SAS programs produce the output by checking for their logic, efficiency and commenting and by review of the produced output.

Syneos Health SOP Pharmacokinetic and Related Data Analysis (3913) describes the process for the interaction between the Biostatistics and Pharmacokinetics departments for the analysis of pharmacokinetic and pharmacodynamic data.