A Phase 1, Randomized, 2-Period, 2-Sequence, Cross-over Study to Determine the Effect of ALXN1840 on the Metabolism of a CYP2B6 Substrate in Healthy Participants

Unique Protocol ID:	ALXN1840-HV-103
NCT Number:	NCT04526210
EudraCT Number:	N/A
Date of SAP:	21 October 2020

Alexion Pharmaceuticals, Inc.

ALXN1840-HV-103

A Phase 1, Randomized, 2-Period, 2-Sequence, Cross-over Study to Determine the Effect of ALXN1840 on the Metabolism of a CYP2B6 Substrate in Healthy Participants

21Oct2020

Final Statistical Analysis Plan

Version 1.0

Prepared by:

PPD
3900 Paramount Parkway
Morrisville, NC 27560 USA

TABLE OF CONTENTS

List of Abbreviations

Abbreviation	Term
AE	adverse event
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_∞	area under the plasma concentration versus time curve from zero to infinity
AUEC	area under the effect versus time curve
$AUEC_t$	area under the effect versus time curve from time 0 to last quantifiable concentration time
BLQ	below the limit of quantification
BMI	body mass index
CI	confidence interval
CL/F	apparent oral clearance
C_{max}	maximum observed plasma concentration
CRF	case report form
CRU	clinical research unit
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
EC	enteric-coated
ECG	electrocardiogram
eDISH	evaluation of drug-induced serious hepatotoxicity
E_{max}	maximum observed PD concentration in plasma
EOS	End of Study
ET	early termination
FSH	Follicle stimulating hormone
GMR	geometric mean ratio
ICF	informed consent form
IQR	interquartile range
MedDRA	Medical Dictionary for Drug Regulatory Activities
MSE	mean-square error
PBC	primary biliary cholangitis
PD	pharmacodynamic
PK	pharmacokinetic(s)
QTcF	QT interval corrected for heart rate using Fridericia's formula
Q1	quartile 1
Q3	quartile 3
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation

Abbreviation	Term
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TE_{max}	time to reach maximum observed pharmacodynamic concentration in plasma
T_{lag}	time delay between the time of dosing and time of appearance of drug concentration in plasma
T_{max}	time to reach maximum observed concentration in plasma
$t_{1/2}$	terminal elimination half-life
V_d/F	apparent volume of distribution
WD	Wilson disease
% AUC_{extrap}	AUC extrapolated from time t to infinity as a percentage of total AUC_∞
λ_z	apparent terminal-phase elimination rate constant

1. Introduction

This document outlines the statistical methods to be implemented during the analysis of data collected within the scope of Alexion Pharmaceuticals, Inc., protocol ALXN1840-HV-103 (A Phase 1, Randomized, 2-Period, 2-Sequence, Cross-over Study to Determine the Effect of ALXN1840 on the Metabolism of a CYP2B6 Substrate in Healthy Participants.), original version, dated 28 Apr 2020. The purpose of this statistical analysis plan is to define the planned statistical analysis of the study data consistent with the study objectives.

ALXN1840 (bis-choline tetrathiomolybdate; formerly known as WTX101) is a novel, first-in-class, copper-protein binding agent in development for the treatment of Wilson disease (WD).

2. Objectives

2.1. Primary Objective

The primary objective of this study is to determine the effect of ALXN1840 on the pharmacokinetics (PK) of bupropion, a sensitive CYP2B6 substrate.

2.2. Secondary Objective

The secondary objectives of this study are to determine the effect of ALXN1840 on the PK of hydroxybupropion, the major active metabolite of bupropion, plasma molybdenum with the coadministration of bupropion, and to determine the safety and tolerability of ALXN1840, with the coadministration of bupropion.

3. Investigational Plan

3.1. Overall Study Design and Plan

The study is being conducted as a randomized, 2-period, 2-sequence, cross-over study to determine the effect of a single dose of ALXN1840 (perpetrator) on the single dose bupropion (victim) kinetics in healthy male and female participants.

The study has a Screening period (Day -28 to Day -2), two 11-day study periods (Day 1 to Day 11) with a minimum of 14 days between doses of bupropion, and an End of Study (EOS) Visit (Day 15 ± 2 days) after Period 2 dosing. Participants will report to the clinical research unit (CRU) on the day prior (Day -1) to both dosing periods.

All participants will receive 1 treatment in each study period; treatment order will be defined based on randomization:

- Treatment A: One 150 mg bupropion HCl salt tablet (WELLBUTRIN SR 150) with 240 mL water (fasting).
- Treatment B: One 150 mg bupropion HCl salt tablet (WELLBUTRIN SR 150) + 4 × 15 mg ALXN1840 EC tablets with 240 mL water (fasting).

Based on randomization, participants will be administered either Treatment A or Treatment B in each period. A wash-out period of at least 14 days must separate dose administration in Period 1 and Period 2. Participants will remain institutionalized from the day prior to dosing until Day 11 following dose administration for each period.

The PK profile of ALXN1840, bupropion and hydroxybupropion will be determined by blood sampling following single dose administration over approximately 5 half-lives or more for both study interventions. Blood sampling for PK assessments will occur at pre-dose, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, and 336 hours post-dose to ensure full assessment of exposure over time. The nominal 336-hour sample for Period 1 is pre-dose sample for Period 2. In addition to PK sampling, safety and tolerability will be assessed by monitoring adverse events (AEs), vital signs, 12-lead electrocardiograms (ECGs), and laboratory parameters.

Following scheduled procedures on Day 11, participants will be discharged from the unit. If there are any participants with clinically significant abnormalities at the time of discharge, the Investigator should notify the Medical Monitor prior to discharge, and participants may be asked to remain institutionalized for further clinical monitoring.

Participants may be asked or required to stay in the CRU between Periods 1 and 2, and/or before the EOS visit, for their own safety, and also to maintain the integrity of the conduct of the study.

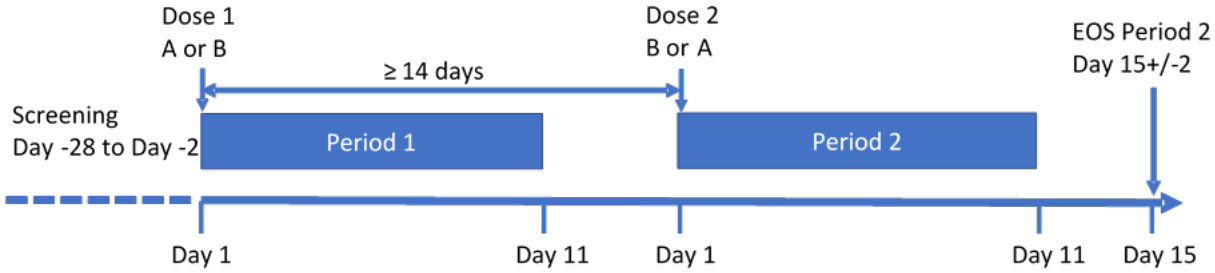
The EOS Visit will occur approximately 4 days after discharge from Period 2, after collection of the nominal 336-hour PK sample.

This study will include approximately 38 participants. Randomization will be stratified by sex.

At the Investigator's discretion, additional participants may be screened to allow for a full enrollment of the study. Therefore, it is possible that all eligible participants may not be included. Participants not included in the study will be discharged without dosing on Day 1.

3.2. Schema

Figure 1: Study ALXN1840-HV-103 Schematic



Participants will be admitted on Day -1 of each period for Check in procedures. Eligible participants will be randomized on Day 1 immediately prior to dosing in Period 1.
Participants will receive treatment (A or B) based on randomization on Day 1 of each period.
Blood samples for PK analysis of total molybdenum (as a measure of ALXN1840) and bupropion will be collected in each period on Day 1 at pre-dose, 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 Day 11. The nominal 336-hour sample for Period 1 will be collected pre-dose in Period 2. Participants will be discharged on Day 11 of each period after completion of all procedures and review of all safety data. The end of study visit (EOS) will occur on Day 15 ± 2 of Period 2, with the collection of 336-hour PK sample for Period 2.
Participants may be asked or required to stay in the CRU between Periods 1 and 2, and/or before the EOS visit, for their own safety, and also to maintain the integrity of the conduct of the study.

3.3. Study Endpoints

3.3.1. Primary Endpoints

The primary endpoints include following PK parameters of bupropion with and without the coadministration of ALXN1840

- C_{max}: maximum observed plasma concentration
- AUC_t: area under the plasma concentration (AUC) versus time curve from time 0 to the last quantifiable concentration
- AUC_∞: AUC versus time curve from time 0 to infinity

3.3.2. Secondary Endpoints

The secondary endpoints include following PK parameters of hydroxybupropion with and without the coadministration of ALXN1840, PK parameters for plasma total molybdenum with the coadministration of bupropion, and safety assessed parameters

- The PK parameters of hydroxybupropion with and without the coadministration of ALXN1840
  - C_{max}

  - AUC_t
  - AUC_∞
- The PK parameters for plasma total molybdenum with the coadministration of bupropion
  - C_{max}
  - AUC_t
  - AUC_∞
- The safety parameters
  - Incidence of treatment-emergent adverse events (TEAEs) and treatment-emergent serious adverse events (TESAEs)
  - Physical examination
  - Vital signs measurements
  - Clinical laboratory results
  - 12-lead ECG results

4. General Statistical Considerations

All data captured on the case report form (CRF), laboratory safety variables, and PK assessments will be listed on an individual basis by treatment sequence and participant. Summary tables will be presented by treatment and overall, unless otherwise specified.

All pre- and post-dose assessments including repeated and/or unscheduled assessments will be included in the data listing.

Continuous variables will be described using the following descriptive statistics: number of non-missing values (n), arithmetic mean, standard deviation (SD), median, quartile 1 (Q1), quartile 3 (Q3), interquartile range (IQR), minimum, and maximum, unless otherwise specified. Categorical variables will be summarized using percentages and frequency count.

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.

Percentages will be suppressed when the count is zero and will be presented to one decimal place. The denominator for all percentages will be the number of participants in the treatment sequence for the population of interest, unless otherwise specified.

Baseline is defined as the last non-missing assessment (including repeated and unscheduled assessments) prior to treatment on Day 1 (dose day for each period), unless otherwise specified. If there are repeated assessments at a time point, the first non-missing

assessment will be included in the summary tables. If the original scheduled measurement at that time point is missing, the next available repeated measurement will be used in the summary tables.

For safety laboratory evaluations, for the purpose of summarization, any numeric values recorded below the lower limit or above the upper limit of quantification will be set to the respective limit for all related summaries. In listings, original results containing inequality sign will be displayed, unless otherwise specified.

Study day will be calculated as follows:

- If assessment date is on or after the dose of treatment, then

  Study Day = Assessment Date –Day 1 Date + 1

- Otherwise,

  Study Day = Assessment Date – Day 1 Date

All statistical analyses detailed in this statistical analysis plan (SAP) will be conducted using SAS® Version 9.4 or higher (SAS Institute, Inc., Cary, North Carolina) and Phoenix® WinNonlin® Version 8.0 or higher (Certara USA, Inc., Princeton, New Jersey).

4.1. Statistical Hypotheses

The primary objective is to determine the effect of ALXN1840 on the PK of bupropion. There is no formal null hypothesis to be statistically tested and used to drive declaration of study success or failure.

To assure the study has enough participants, to estimate the inhibitory effect of ALXN1840 on the metabolism of bupropion with adequate precision, the sample size was determined using the conservative 90% confidence interval (CI) and 80% to 125% no-effect boundary approach described in the FDA Guidance "Clinical Drug Interaction Studies —Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions" (2020). This guidance notes the 80 to 125 % boundaries represent a very conservative standard for drugs that have wide safety "margins" and "the totality of evidence should be taken into consideration when determining the clinical impact of the DDI on the substrate drug". Therefore, if the estimated inhibition effect of ALXN1840 on bupropion PK lies outside this no-effect boundary, this should not be interpreted as a failed study.

4.2. Sample Size

A default of no-effect boundary (80% to 125%) approach will be employed to assess whether there is an inhibition effect on the metabolism of bupropion in the presence of ALXN1840. For a 2-period crossover study design, assuming the true ratio of the means (bupropion + ALXN1840 vs bupropion alone) on the PK parameters is 1 (C_{max}; AUC_{∞}) and the bupropion intra-participant coefficient of variation (CV) is 0.30 (Dennison, 2018), a total sample size of 32 participants can achieve 90% power with two 1-sided tests, each with a type I error rate of 5% (two-sided type I error rate of 10%). The intra-participant CV

is estimated using residual mean-square error (MSE) term in the analysis of variance model. The CV is estimated as 100% × √ (exp (MSE) - 1) for the log-transformed PK parameters. Assuming a 15% dropout rate, approximately 38 participants will be enrolled.

4.3. Randomization, Stratification, and Blinding

This is a randomized, open-label, 2-period, 2-sequence, cross-over study. The randomization will be stratified by sex.

4.4. Analysis Set

4.4.1. Enrolled Set

The enrolled set will include all participants who sign the informed consent form (ICF).

4.4.2. Pharmacokinetic/Pharmacodynamic Analysis Set

The PK analysis set will include all participants who have sufficient plasma samples to have evaluable PK data for bupropion and/or total molybdenum (as a measure of ALXN1840) in plasma.

The Pharmacodynamic (PD) analysis set will include all participants who have sufficient plasma samples to have evaluable PD data for total copper or PUF copper.

4.4.3. Safety Set

The safety set will include all participants who receive at least 1 dose of study intervention.

5. Participant Disposition

5.1. Disposition

Using the enrolled set, the screen failure of participants will be summarized with counts and percentages by overall. Summary table will include the following:

- Number of participants in the enrolled set (ie, all participants who signed the ICF)
- Number of participants who were screen failures
- Number of participants who were randomized
- Number of participants who failed screen due to COVID-19 related reasons
- Reason for screen failure that was not due to eligibility

Based on the randomized participants, the analysis sets will be summarized with counts and percentages by treatment and overall. Summary table will include the following:

- Number of participants included in PK/PD analysis set
- Number of participants excluded from the PK/PD analysis set

- Reason for exclusion from the PK/PD analysis set
- Number of participants included in safety set
- Number of participants excluded from the safety set
- Reason for exclusion from the safety set

Using the randomized participants, PK analysis set, and safety set respectively, the disposition of participants will be summarized with counts and percentages by treatment and overall. Summary table will include the following:

- Number of participants who completed the study
- Number of participants who discontinued the study
- Reasons for discontinuation
- Number of participants who discontinued the treatment
- Reasons for treatment discontinuation
- Number of participants who were discontinued due to COVID-19

Screen failure data, participant disposition data, and analysis set will be presented in separate data listings for the participants who randomized.

5.2. Protocol Deviations

The protocol deviations will be summarized by treatment and overall for the safety set.

All protocol deviations will be presented in a data listing based on randomized participants.

6. Demographics and Baseline Characteristics

6.1. Demographics and Baseline Characteristics

Age, sex, race, ethnicity, height, and body mass index (BMI) will be collected at Screening; weight will be collected at Screening and Check-in for each period.

Demographic characteristics including age, sex, race, ethnicity, weight (kg), height (cm), and BMI (kg/m^2) will be summarized by treatment sequence and overall for the safety set.

Weight measurements collected at Check-in to Dosing Period 1 will be used as baseline for the summary.

Demographic characteristics will be presented in a data listing for the safety set.

6.2. Urine Alcohol and Drug screen

Urine alcohol test and urine drug screen will be performed at Screening and Check-in for each period.

Urine alcohol and drug screen results in the safety set will be presented in a data listing for the safety set.

6.3. Medical History

Medical history will be collected at Screening.

Medical history results will be presented in a data listing for the safety set with each verbatim term be mapped to a system organ class and preferred term using the Medical Dictionary for Drug Regulatory Activities (MedDRA, Version 23.0 or later).

6.4. Inclusion and Exclusion Criteria

Inclusion and exclusion criteria will be confirmed at Screening and Check-in for each period.

Admission criteria deviations are defined as any violation of protocol-defined inclusion/exclusion criteria.

Admission criteria deviations will be presented in a data listing for the participants who randomized.

6.5. Genetics

Blood samples for genetic testing of CYP2B6 polymorphism will be collected at clinical Check-in (Day -1) for Period 1.

Genetic testing results will be listed and summarized by treatment sequence and overall for the safety set.

7. Treatments and Medications

7.1. Prior and Concomitant Medications

Prior medications are defined as medications that were started and ended prior to the first dose of treatment. Concomitant medications are defined as medications that were administered after the first dose of treatment. Medications initiated prior to the first dose of treatment and were continued after the first dose of treatment will be counted as both prior and concomitant medications.

Prior concomitant medications will be summarized by treatment sequence and overall for the safety set. Concomitant medications will be summarized by treatment and overall for the safety set. The anatomical therapeutic chemical code coding scheme of the World Health Organization Drug Dictionary Version March 2019 or later will be used to group medications into relevant categories for the tabulation.

All prior and concomitant medications will be presented in a data listing for the safety set.

7.2. Non-Pharmacologic Therapies and Procedures

Non-pharmacologic therapies and procedures will be coded using the MedDRA Version 23.0 or later and will be presented in a data listing for the safety set.

7.3. Study Treatments

Study treatments will be administered in each study period at the morning of Day 1 and all participants will receive 1 treatment in each study period. Study treatments are as follow

- Treatment A: One 150 mg bupropion HCl salt tablet (WELLBUTRIN SR 150) with 240 mL water (fasting).
- Treatment B: One 150 mg bupropion HCl salt tablet (WELLBUTRIN SR 150) + 4 × 15 mg ALXN1840 EC tablets with 240 mL water (fasting).

Any dose of ALXN1840 or bupropion greater than that specified in the protocol will be considered an overdose.

Study treatment administration data will be presented in a data listing for the safety set.

8. Safety Analysis

All safety analyses will be performed on the safety set.

Safety analyses will include an analysis of all AEs, ECGs, clinical laboratory data, physical examinations, and vital sign measurements using descriptive statistics. Summaries will be presented by treatment and overall, unless otherwise stated. Shift tables will be summarized with count and percentages of participants in each category. Percentages will be based on the number of participants with both non-missing baseline and relevant post-baseline results.

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

8.1. Adverse Events

An AE is any untoward medical occurrence in a participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Adverse events will be analyzed in terms of TEAEs which are defined as any AEs that begin or worsen on or after the first dose of treatment until the EOS or early termination (ET). If the onset date/time of an AE is missing and AE end date is on or after the first dose of treatment, the AE will be defined as treatment-emergent.

All AEs will be listed from the time the participant signs ICF until the EOS or ET Visit.

A treatment-related TEAE is defined as TEAE which was considered to be related to the treatment.

All AEs will be coded by system organ class and PT using the MedDRA Version 23.0 or later.

All summary tables will include number and percentage of participants and number of events, unless otherwise stated. For the number of AEs, each occurrence will be counted once. Percentages will be based upon the number of participants in the safety set.

In the summary tables where both system organ class and preferred term are presented, the default ordering of system organ class will be alphabetical and the default ordering for preferred term will be most prevalent (using percentage) preferred term within each system organ class based on Total column. For PTs with the same number of participants, they should be further sorted by number of events and then alphabetically.

In the by- participant analyses, a participant having the same event more than once within the same level of summarization will be counted only once within the same treatment using the most intense or worst highest event (for by toxicity grade summary) or the most related event (for by relationship summary).

In summaries of AEs by treatment, AEs will be summarized according to the most recent treatment received prior to the AE onset, regardless of when the AE ended. For example, an AE that started on or after administration of the treatment on Day 1 in Dosing Period 1 but before the administration of treatment on Day 1 in Dosing Period 2 will be summarized for the treatment in Period 1; an AE that started on or after administration of treatment on Day 1 in Dosing Period 2 will be summarized for the treatment in Period 2. The AEs that started before administration of treatment on Day 1 in Dosing Period 1 will not be summarized unless if the AE meets the TEAE definition, in that case, the AEs will be summarized for the treatment received prior to the AEs worsened.

8.1.1. Incidence of Adverse Events

An overview summary of TEAEs will be provided and will include the following

- Participants with at least 1 TEAE
- Participants with at least 1 TEAE related to study drug
- Participants with at least 1 TESAE
- Participants with at least 1 TESAE related to study drug
- Participants who drug withdrawn due to TEAE
- Participants who drug withdrawn due to TEAE related to study drug

The frequency of TEAEs will be tabulated by system organ class and preferred term.

8.1.2. Relationship of Adverse Events to Study Drug

The relationship between the study intervention and each occurrence of each AE or SAE is assessed by the Investigator. The relationship will be classified as Related or Not Related.

The frequency of TEAEs will be tabulated by system organ class, PT and by relationship.

8.1.3. Intensity of Adverse Event

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories from National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v5.0, published 27 Nov 2017:

- Grade 1: Mild (awareness of sign or symptom, but easily tolerated)
- Grade 2: Moderate (discomfort sufficient to cause interference with normal activities)
- Grade 3: Severe (incapacitating, with inability to perform normal activities)
- Grade 4: Life-threatening
- Grade 5: Fatal

The frequency of TEAEs will be tabulated by system organ class and preferred term and by intensity.

8.1.4. Serious Adverse Events

Serious adverse events (SAEs) will be analyzed in terms of TESAEs which are defined as any SAEs that begin or worsen on or after the first dose of treatment until the EOS or ET.

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires participant hospitalization or prolongation of existing hospitalization
- Results in persistent disability/incapacity
- Is a congenital anomaly or birth defect
- Other situations (such as important medical events)

The frequency of TESAEs will be tabulated by system organ class and preferred term, and by relationship.

Treatment-emergent SAEs will be listed separately.

8.1.5. Adverse Events Leading to drug withdrawal

Treatment-emergent AEs leading to drug withdrawal will be listed separately.

8.2. Clinical Laboratory Evaluations

Laboratory assessments (including chemistry, hematology, and coagulation) will be performed at Screening, Check-in for each period, Day 2 for each period, Day 5 for each period, Day 10 for each period, and EOS or ET.

Urinalysis will be performed at Screening and Check-in for each period.

Summary statistics and change from baseline for chemistry, hematology, and coagulation will be presented by treatment and by visit. Laboratory parameter values will be graded according to the National Cancer Institute CTCAE. Shift tables will be produced for chemistry, hematology, and coagulation. Shift from baseline grade relative to the reference ranges tables will be presented by treatment and by visit. Shift from baseline to post-baseline worst highest grade will be presented by treatment and study period. Last recheck values collected prior the dose of treatment will be used as baseline and all rechecks will be deleted from post-dose observations in calculating summary statistics.

All clinical laboratory tests (including chemistry, hematology, coagulation, and urinalysis) will be presented in data listings.

8.2.1. Chemistry

Serum chemistry evaluation will include blood urea nitrogen, potassium, bicarbonate, sodium, glucose, aspartate aminotransferase, alanine aminotransferase, chloride, alkaline phosphatase, direct bilirubin, total bilirubin, albumin, creatinine, and creatine phosphokinase.

8.2.2. Hematology

Blood hematology evaluation will include platelet count, red blood cell (RBC) count, hemoglobin, hematocrit, RBC indices (including mean corpuscular volume, mean corpuscular hemoglobin, and % reticulocytes), and white blood cell count with differential (including neutrophils, lymphocytes, monocytes, eosinophils, and basophils).

8.2.3. Coagulation

Blood coagulation evaluation will include international normalized ratio, partial thromboplastin time, and prothrombin time.

8.2.4. Urinalysis

Urinalysis evaluation will include specific gravity, pH, glucose, protein, blood, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick, and microscopic examination.

8.3. Vital Sign Measurements

Oral temperature, pulse rate, respiratory rate, and systolic and diastolic blood pressure (mm Hg) will be performed at Screening, Day 1 (Pre-dose, 1 Hour, 4 Hour, 8 Hour, and 12 Hour) for each period, Day 2 (24 Hour) for each period, and EOS or ET.

Weight will be collected at Screening and Check-in for each period. Height and BMI will be collected at Screening only.

Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (eg, television, cell phones).

Summary statistics and change from baseline for vital signs (temperature, pulse rate, respiratory rate, and systolic and diastolic blood pressure) will be presented by treatment and overall and by visit and time point. Last recheck values collected prior to the dose of treatment will be as baseline and all rechecks will be deleted from post-dose observations in calculating summary statistics.

Vital sign measurement results, including height, weight, and BMI, will be presented in a data listing.

8.4. Physical Examination

A full physical examination, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, and neurological systems will be performed at Screening, Check-in for each period, and EOS or ET. A symptom-driven physical examination may be performed at other times, at the principal investigator's discretion.

General appearance, skin, head/ear/eye/nose/throat, neck, lymph node, chest, heart, abdominal cavity, limb, central nervous system, and musculoskeletal will be captured on the CRF.

Physical examination results will be presented in a data listing.

8.5. Electrocardiogram

Triplicate 12-lead ECG will be performed at Screening and Day 1 (Pre-dose and 4 Hour [before lunch]) for each period.

Participants will be resting in the supine position for at least 15 minutes prior to and 5 minutes after each nominal time point for ECG extraction.

Heart rate, PR interval, QRS interval, RR interval, QT interval, QT interval corrected for heart rate using Fridericia's formula (QTcF), and interpretation of ECG will be captured on the CRF.

The average of the triplicate ECG readings at the time points collected will be calculated. Summary statistics and change from baseline of calculated average for heart rate, PR interval, QRS interval, RR interval, QT interval, and QTcF interval will be presented by time point. Last recheck values collected prior to the dose of treatment will be as baseline

and all rechecks will be deleted from post-dose observations in calculating summary statistics.

Triplicate 12-lead ECG findings will be presented in a data listing.

8.6. Other Safety Data

8.6.1. Liver Function Results

All events of ALT $\geqslant 3 \times$ upper limit of normal (ULN) and bilirubin $\geqslant 2 \times$ ULN (> 35% direct bilirubin) or ALT $\geqslant 3 \times$ 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 (excluding studies of hepatic impairment or cirrhosis).

Liver function results will be presented in a summary table and eDISH (evaluation of drug-induced serious hepatotoxicity) plots.

8.6.2. Serology

Serology including HIV, hepatitis B, and hepatitis C screen will be performed at Screening.

Serology results will be presented in a data listing.

8.6.3. Pregnancy Test

Serum or urine pregnancy test for female participants will be performed at Screening, Check-in for each period, and EOS or ET.

Pregnancy test results will be presented in a data listing.

8.6.4. Follicle Stimulating Hormone

Follicle stimulating hormone (FSH) will be performed at Screening and only needed if claiming exemption from contraception requirement due to menopause.

FSH results will be presented in a data listing.

8.6.5. Discussion/Documentation of Contraception Method

Discussion/documentation of contraception method will be performed at Screening and Check-in for each period. The discussion/documentation of contraception method results will be kept in the source documents and will not be recorded on the CRF; therefore, the discussion/documentation of contraception method results will not be presented in any of the tables, listings, or figures.

8.6.6. Retained Sample

A single 12 mL sample will be retained for evaluation in the event of an unexpected safety finding. The retained serum sample will only be collected pre-dose on Day 1 of Period 1.

Retained sampling results will be presented in a data listing.

9. Pharmacokinetics and Pharmacodynamic Analysis

9.1. Pharmacokinetic Sampling

Whole blood samples will be collected for the measurement of plasma concentrations of total molybdenum, PUF molybdenum, bupropion and hydroxybupropion at the following time points: pre-dose and post-dose at 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, and 336 hours in each period. Additional samples may be collected at additional time points during the study if warranted and agreed upon between the Investigator and Alexion. Collection of samples for PK evaluation should occur as close as possible to the scheduled time and actual time of collection should be documented on the eCRF. Samples collected within ± 10% of the scheduled time, or 30 minutes, whichever is less, will not be considered a protocol deviation. In the event of a safety occurrence and after agreement between the Investigator and Medical Monitor, up to 3 additional PK sampling timepoints may be added.

PK samples will also be used for the PD analysis of total copper and PUF copper.

9.2. Below the Limit of Quantification Values / Missing Values

For PK and the PD measure plasma total copper parameter calculations, concentrations below the limit of quantification (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.

For PK and PD concentration summaries, BLQ values will be set to zero.

9.3. Pharmacokinetic/Pharmacodynamic Data Presentation Conventions

PK and PD concentration data will be summarized using descriptive statistics (number of participants (N), number of participants with available data (n), arithmetic mean, SD, arithmetic CV, geometric mean (GM), geometric CV, median, minimum and maximum).

The predose PD concentration will be used as baseline; absolute change from baseline will be calculated as: a postdose concentration value – baseline concentration value; percent change from baseline will be calculated as: ([a postdose concentration value – baseline concentration value] / baseline concentration value) ×100%.

The following conventions will be applied to PK/PD presentations and summaries.

- For continuous variables, all mean and median values are formatted to one more decimal place than the measured value. Standard deviation values are formatted to two more decimal places than the measured value. Minimum and maximum values are presented with the same number of decimal places as the measured value.

- Date variables are formatted as DDMMMYYYY for presentation. Time is formatted in military time as HH:MM for presentation.

9.4. Pharmacokinetic Parameters

The following plasma PK parameters will be calculated for total molybdenum, PUF molybdenum, bupropion and hydroxybupropion 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. It is expected that in Treatment B the dosing of ALXN1840 and bupropion will occur at the same time. Calculations will be based on the actual sampling times relative to the actual reference ALXN1840 or bupropion dosing times recorded during the study.

- T_{lag}: time delay between the time of dosing and time of appearance of drug concentration in plasma (for plasma total molybdenum and PUF molybdenum with ALXN1840 administration)
- C_{max}: maximum observed concentration in plasma
- T_{max}: time to reach maximum observed concentration in plasma
- AUC_t: AUC from time 0 to the last quantifiable concentration, calculated using the linear trapezoidal rule
- AUC_∞: AUC versus time curve from time 0 to infinity, calculated using the linear trapezoidal rule
- % AUC_{extrap}: AUC extrapolated from time t to infinity as a percentage of total AUC_∞
- $t_{1/2}$: terminal elimination half-life
- λ_z: apparent terminal-phase elimination rate constant

For ALXN1840, equivalent molybdenum dose will be used to calculate total molybdenum (and PUF molybdenum if applicable) CL/F and V_d/F values: molybdenum MW of 95.95 Da/ALXN1840 MW of 432.54 Da × 60 mg ALXN1840 = 13.31 mg molybdenum dose. For bupropion, 150 mg dose will be used for the calculation.

- CL/F: apparent oral clearance
- V_d/F: apparent volume of distribution

For hydroxybupropion, metabolite to parent ratios will be reported for C_{max}, AUC_t and AUC_∞.

Additional plasma PK parameters may be calculated if deemed appropriate.

9.5. Pharmacokinetic Statistical Analysis

Plasma concentration data for total molybdenum, PUF molybdenum, bupropion and hydroxybupropion will be summarized separately for each scheduled sampling time using descriptive statistics. Plasma concentrations of total molybdenum, PUF molybdenum, bupropion and hydroxybupropion and time deviation data will be presented in a data listing by participant. 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.

Pharmacokinetic parameters derived from plasma concentrations of total molybdenum, PUF molybdenum, bupropion and hydroxybupropion will be presented in data listings and summarized separately using the following descriptive statistics: N, n, arithmetic mean, SD, arithmetic CV, median, minimum, maximum, and 95% confidence interval (CI). Geometric mean and geometric CV will be presented for C_{max} and AUCs only.

The effect of ALXN1840 on bupropion and hydroxybupropion PK will be assessed using a linear mixed-effect model (SAS PROC MIXED) with treatment condition, sequence, and period as fixed effects and participant nested within sequence as a random effect. The model will be fitted to the natural-log-transformed PK parameters C_{max}, AUC_t, and AUC_∞ for estimation of effects and construction of CIs for the test treatment (Treatment B: 150 mg bupropion + 60 mg ALXN1840) compared with the reference treatment (Treatment A: 150 mg bupropion). The within-participant CV for the corresponding PK parameters will be estimated using the mean squared error from the statistical model. Confidence intervals (90%) will be constructed for the least-squares geometric mean ratio (GMR) estimates between the test and reference treatments for the indicated PK parameters using the natural log-transformed data. The GMR estimates and the associated 90% confidence limits will be exponentiated back to the original scale. The statistical results will also be graphically presented using a forest plot.

9.6. Genetic Variant Analyses for CYP2B6

Participants with variant CYP2B6 alleles will be identified in the PK concentration and parameter listings, where applicable. Characterization of the potential impact of ALXN1840 on phenotypic CYP2B6 metabolism of bupropion to the formation of hydroxybupropion and genetic polymorphism on the between-participant variability of bupropion PK are exploratory assessments. Thus, this study does not specifically select target participants with a certain proportion of CYP2B6 genotypes. Depending on the distribution of CYP2B6 genotypes within treatments, the impact of ALXN1840 coadministration on bupropion and hydroxybupropion PK may be stratified by CYP2B6 genotypes to assess the potential impact of ALXN1840 on bupropion metabolism.

9.7. Pharmacodynamic Parameters

The following PD parameters will be calculated using noncompartmental methods for plasma total copper and PUF copper (measured and absolute change from baseline)

concentrations 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 actual sampling times relative to the actual reference ALXN1840 dosing times recorded during the study.

- E_{max}: maximum observed PD concentration in plasma
- TE_{max}: time to reach maximum observed PD concentration in plasma
- $AUEC_t$: area under the effect versus time curve (AUEC) from time 0 to last quantifiable concentration time, calculated using the linear trapezoidal rule

Additional PD parameters may be calculated as necessary.

9.8. Pharmacodynamic Statistical Analysis

Plasma concentration data for total copper and PUF copper will be summarized separately for each scheduled sampling time using descriptive statistics. Individual concentration data will be presented in data listings. Individual concentration versus actual time profiles will be provided. Additionally, mean concentration versus nominal time profiles will be provided. Note that the measured, change from baseline, and percent change from baseline concentration data for total copper and PUF copper will be listed, summarized, and presented graphically on linear scales.

Parameters for total copper and PUF copper in plasma will be summarized separately using descriptive statistics (N, n, mean, SD, median, %CV, minimum, and maximum). Geometric mean and geometric %CV will be calculated for AUECs and E_{max}.

10. Interim Analysis

No interim analyses are planned for this study.

11. Changes in the Planned Analysis

For the definition of PK/PD analysis set in the protocol was “All participants who have sufficient plasma samples to have evaluable PK data for bupropion and total molybdenum (as a measure of ALXN1840) in plasma”, in this SAP is changed to “The PK analysis set will include all participants who have sufficient plasma samples to have evaluable PK data for bupropion and/or total molybdenum (as a measure of ALXN1840) in plasma. The Pharmacodynamic (PD) analysis set will include all participants who have sufficient plasma samples to have evaluable PD data for total copper or PUF copper.”

The protocol required “the overall study population will include a minimum of 40% of each sex” and “there will be no less than 16 and no more than 22 of either sex (ie, a maximum split in either direction of approximately 60%:40%)”, but in the Administrative Change Letter 2 (dated 09 September 2020) required to remove the 60%:40% male/female ratio requirement. So, in this SAP, the 60%:40% male/female ratio for the participant randomization is not required.

12. References

Dennison J, Puri A, Warrington S, Endo T, Adeloye T, Johnston A. Amenamevir: Studies of Potential CYP2C8- and CYP2B6-Mediated Pharmacokinetic Interactions With Montelukast and Bupropion in Healthy Volunteers. Clin Pharmacol Drug Dev. 2018;7(8):860-870.

PPD	PPD Biostatistics and Programming
	Statistical Analysis Plan (SAP) Client Approval Form

Client:	Alexion Pharmaceuticals, Inc.
Protocol Number:	ALXN1840-HV-103

Document Description:	Final Statistical Analysis Plan
SAP Title:	A Phase 1, Randomized, 2-Period, 2-Sequence, Cross-over Study to Determine the Effect of ALXN1840 on the Metabolism of a CYP2B6 Substrate in Healthy Participants
SAP Version Number:	Version 1.0
Effective Date:	21 Oct 2020

Author(s):

For PPD: PPD Pharmacokineticist

For PPD: PPD Biostatistician

Approved by:

PPD

26-Oct-2020 | 12:56:14 EDT

PPD

Date *(DD-MMM-YYYY)*

AD, Biost

Alexion Pharmaceuticals, Inc.

PPD

26-Oct-2020 | 13:29:29 EDT

PPD

Date *(DD-MMM-YYYY)*

Senior Dir es

Alexion Pharmaceuticals, Inc.

PPD [redacted]

26-Oct-2020 | 13:55:10 EDT

PPD [redacted]

Date *(DD-MMM-YYYY)*

Senior

Alexion

PPD [redacted]

26-Oct-2020 | 13:14:11 EDT

PPD [redacted]

Date *(DD-MMM-YYYY)*

Senio [redacted] Pharmacology

Alexion Pharmaceuticals, Inc.

Certificate Of Completion

Envelope Id: 9D6148BBBB2243D1B3975820A2ACAF68
Status: Completed
Subject: Please DocuSign: ALXN1840-HV-103 SAP Approval Form.pdf
Source Envelope:
Document Pages: 2 Signatures: 4
Certificate Pages: 2 Initials: 0
AutoNav: Enabled
EnvelopeId Stamping: Disabled
Time Zone: (UTC-05:00) Eastern Time (US & Canada)

Envelope Originator:
PPD
PPD
New Haven, CT 06510
PPD
IP Address: PPD

Record Tracking

Status: Original
26-Oct-2020 | 12:47
Holder: PPD
PPD
Location: DocuSign

Signer Events	Signature	Timestamp
PPD PPD Director, Global Medical Sciences Alexion Pharmaceuticals, Inc. Security Level: Email, Account Authentication (Required)	PPD Signature Adoption: Pre-selected Style Signature ID: PPD Using IP Address: PPD With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 26-Oct-2020 | 12:55 Viewed: 26-Oct-2020 | 13:29 Signed: 26-Oct-2020 | 13:29
Electronic Record and Signature Disclosure: Not Offered via DocuSign		
PPD PPD Assoc. Director, Biostatistics Alexion Pharmaceuticals Inc. Security Level: Email, Account Authentication (Required)	PPD Signature Adoption: Pre-selected Style Signature ID: PPD Using IP Address: PPD With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 26-Oct-2020 | 12:55 Viewed: 26-Oct-2020 | 12:55 Signed: 26-Oct-2020 | 12:56
Electronic Record and Signature Disclosure: Not Offered via DocuSign		

Signer Events	Signature	Timestamp
PPD PPD Director, Biostatistics Alexion Pharmaceuticals, Inc. Security Level: Email, Account Authentication (Required)	PPD Signature Adoption: Pre-selected Style Signature ID: PPD Using IP Address: PPD With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 26-Oct-2020 | 12:55 Viewed: 26-Oct-2020 | 13:54 Signed: 26-Oct-2020 | 13:55
Electronic Record and Signature Disclosure: Not Offered via DocuSign		
PPD PPD Senior Director, Clinical Pharmacology Alexion Pharmaceuticals, Inc. (Regulated) Security Level: Email, Account Authentication (Required)	PPD Signature Adoption: Pre-selected Style Signature ID: PPD Using IP Address: PPD With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 26-Oct-2020 | 12:55 Viewed: 26-Oct-2020 | 13:13 Signed: 26-Oct-2020 | 13:14
Electronic Record and Signature Disclosure: Not Offered via DocuSign		

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	26-Oct-2020 | 12:55
Certified Delivered	Security Checked	26-Oct-2020 | 13:54
Signing Complete	Security Checked	26-Oct-2020 | 13:55
Completed	Security Checked	26-Oct-2020 | 13:55

Payment Events	Status	Timestamps