A Randomized, Three-Treatment, Three-Period, Six-Sequence, Crossover, Placebo- and Active-Controlled, Double-Blind for ALXN1840 (Open-Label for Moxifloxacin) Thorough QT/QTc (TQT) Study to Evaluate the Effect of ALXN1840 on Cardiac Repolarization in Healthy Adult Participants

Unique Protocol ID:	ALXN1840-HV-107
NCT Number:	NCT04560816
Date of SAP:	03 September 2020

STATISTICAL ANALYSIS PLAN
PHASE I

DATE OF PLAN:

03-Sep-2020

BASED ON:

Protocol Amendment 1

STUDY DRUG:

ALXN1840

PROTOCOL NUMBER:

ALXN1840-HV-107

STUDY TITLE:

A Randomized, Three-Treatment, Three-Period, Six-sequence, Crossover, Placebo- and Active-Controlled, Double-Blind for ALXN1840 (Open-Label for Moxifloxacin) Thorough QT/QTc (TQT) Study to Evaluate the Effect of ALXN1840 on Cardiac Repolarization in Healthy Adult Participants

SPONSOR:

Alexion Pharmaceuticals, Inc.

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Date

03-Sep-2020 | 14:58:30 EDT

Date

TECHNICAL SUMMARY REPORT

<table>
<tr><td>Name of Sponsor/Company:
Alexion Pharmaceuticals, Inc.</td><td>Individual Study Table Referring to Part of the Dossier:
Volume:</td><td>(For National Authority Use Only):</td></tr>
<tr><td>Name of Finished Product:
ALXN1840</td><td>Page:</td><td></td></tr>
<tr><td>Name of Active Ingredient:
Bis-choline tetrathiomolybdate</td><td></td><td></td></tr>
<tr><td colspan="3">Title of Study:
A Randomized, Three-Treatment, Three-Period, Six-Sequence, Crossover, Placebo- and Active-Controlled, Double-Blind for ALXN1840 (Open-Label for Moxifloxacin) Thorough QT/QTc (TQT) Study to Evaluate the Effect of ALXN1840 on Cardiac Repolarization in Healthy Adult Participants</td></tr>
<tr><td colspan="3">Investigators:
Study Center(s): One clinical site in the United States</td></tr>
<tr><td>Studied period:
Duration of participant participation from the Screening Visit to the End-of-Study (EoS) Visit will be approximately 70 days</td><td colspan="2">Phase of development:
Phase 1</td></tr>
<tr><td colspan="3">Objectives:
Primary:
The primary objective of this study is to evaluate the effect of a supratherapeutic dose of ALXN1840 on the heart rate (HR)-corrected QT interval (QTc) with the intent to exclude a 10 ms effect.
Secondary: The secondary objectives of the study are:
• To demonstrate assay sensitivity of the study to detect an effect on the mean QT/QTc interval of approximately 5 ms using moxifloxacin as a positive control.
• To evaluate the safety and tolerability of a single oral supratherapeutic dose of ALXN1840 in healthy participants.
• To assess the pharmacokinetics (PK) of ALXN1840 following administration of a single oral supratherapeutic dose in healthy participants.
• To evaluate the effects of a single oral supratherapeutic dose of ALXN1840 on HR, PR and QRS intervals, treatment-emergent T-wave morphology abnormalities, and appearance of U waves.

Exploratory: The exploratory objective of this study is to evaluate the effects of plasma total molybdenum (Mo) and plasma ultrafiltrate (PUF) Mo (as a surrogate measure of ALXN1840) plasma concentrations on QT/QTc.</td></tr>
</table>

Methodology:
This is a randomized, 3-treatment, 3-period, 6-sequence, crossover, placebo- and active-controlled, double-blind for ALXN1840, open-label for moxifloxacin, in healthy adult participants. Moxifloxacin will be used as the active control.

Study interventions will be given in randomized sequences: ABC, ACB, BAC, BCA, CAB, and CBA, with each treatment administered with 240 mL of water following an overnight fast of at least 10 hours. No food will be allowed for at least 4 hours postdose. Water can be allowed as desired except for 1 hour before and after drug administration. Treatment A is a single oral dose of ALXN1840 120 mg administered as 15-mg enteric-coated tablets (supratherapeutic dose); Treatment B is a single oral dose of enteric-coated placebo tablets matching ALXN1840; and Treatment C is a single oral dose of moxifloxacin 400 mg tablet.

Cardiodynamic assessment will be performed for approximately 25 hours on Day –1 of Treatment Period 1 and Day 1 to Day 2 of each treatment period. Blood samples for PK will be collected predose and at specified times up to 96 hours after the Day 1 dose in each treatment period.

Twelve-lead electrocardiograms (ECGs) will be extracted from a continuous (Holter) recording by the central laboratory. To support high quality data for extraction, 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. Blood for plasma PK will be collected as close as possible to nominal time but after completion of ECG extraction with actual times for blood sample collections recorded.

Participants will be domiciled in the clinic for 7 days in Treatment Period 1 and for 6 days in Treatment Periods 2 and 3, starting on Day –2 for Treatment Period 1 and on the day before dosing (Day –1) for Treatment Periods 2 and 3, until safety procedures have been completed and reviewed on Day 5.There will be a washout of at least 14 days between dose administration in each period.

All participants (including participants who terminate the study early) will return to the study site 14 days (±2 days) after the last administration of study intervention for follow-up procedures and to determine if any adverse event (AE) has occurred since the last study visit.

Duration of participant participation from the Screening Visit to the End-of-Study Visit will be approximately 70 days. This includes up to 29 days for the Screening Period, study intervention administration on Day 1 of each treatment period, a minimum of a 14-day washout between study intervention administration in each treatment period, and 14 days (±2 days) for the EOS Visit.

Number of Participants (planned and analyzed):
Approximately 54 participants will be randomized to ensure there will be 45 evaluable participants with data from all treatment periods. To ensure sequences are balanced within sex, participant randomization will be stratified by sex.

Diagnosis and main criteria for inclusion:

- Participant is a healthy adult male or female, 18 to 50 years of age, inclusive, at Screening.
- Participant is a continuous nonsmoker and or an individual who has not used tobacco or nicotine-containing products (eg, electronic vapor cigarettes, cigarettes, cigars, chewing tobacco, snuff, nicotine gum, nicotine patches) for at least 3 months prior to the first dose of study intervention and for the duration of the study. A cotinine test performed during Screening and Check-in must be consistent with continuous nonsmoking status.
- Participant must weigh at least 60 kg for males or 52 kg for females and have a body mass index ≥18.0 and ≤30.0 kg/m2 at Screening.
- Participant must have a medical assessment with no clinically significant or relevant abnormalities as determined by medical history, physical examination, vital signs, 12-lead ECG, and clinical laboratory evaluation (hematology, serum chemistry, coagulation, and urinalysis) that are reasonably likely to interfere with the participant's participation in or ability to complete the study, or to potentially confound interpretation of study results, as assessed by the Investigator.
- Participant has no clinically significant history or presence of ECG findings as judged by the Investigator at Screening and Check-in

Test product, dose and mode of administration, batch number:
A single oral dose of ALXN1840 120 mg administered as 15-mg enteric- coated tablets (supratherapeutic)

Duration of treatment:
The study consists 3 treatment periods, study intervention administration on Day 1 of each treatment period, a minimum of a 14-day washout between study intervention administration in each treatment period, and 14 days (±2 days) for the EOS Visit.

Reference therapy, dose and mode of administration, batch number:
A single oral dose of enteric-coated placebo tablets matching ALXN1840
A single oral dose of moxifloxacin 400 mg tablet

Criteria for evaluation:
Cardiodynamic Assessments and Endpoints:

Twelve-lead ECGs will be extracted from approximately 25-hour continuous (Holter) recordings on Day –1 of Treatment Period 1 and on Days 1 and 2 in each treatment period. Participants should be resting supinely for at least 15 minutes before and 5 minutes after each time point for ECG extraction, which should precede PK blood samples, when applicable.

Electrocardiograms will be extracted from the continuous recording by a central ECG laboratory (ERT, Rochester, NY) on Day –1 of Treatment Period 1 and each of these days at the following time points: Day 1 at predose (–45, –30, and –15 minutes) and 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 (Day 2) hours postdose, for a total of 15 (3 predose and 12 postdose) time points in each treatment period. For HR-corrected QTc calculation in case of a substantial HR effect due to ALEXN40, ECG extraction from the Holter recording Day -1 of Treatment Period 1 will include continuous ECG extraction for optimized QT interval (QTcI) calculation, and if needed, ECG extraction at timepoints that precisely match the extraction timepoints for Day 1 of Periods 1, 2 and 3 for individual HR-corrected QT interval (QTcS) calculation.

The following ECG parameters will be measured and calculated: HR, PR interval, QT interval corrected for heart rate (QTc), QRS interval, treatment-emergent T-wave morphology abnormalities, and appearance of U-waves. The primary endpoint is placebo-corrected change from Baseline QTc (ΔΔQTc, correction method will be data driven) for ALXN1840 using the by-time point analysis. The secondary endpoints are the ΔΔQTc for moxifloxacin; the change from Baseline in HR, QTc, PR, QRS (ΔHR, ΔQTc, ΔPR and ΔQRS); the placebo-corrected change from Baseline HR, PR, and QRS (ΔΔHR, ΔΔPR and ΔΔQRS); categorical outliers for QTc, HR, PR, and QRS; and the frequency of treatment-emergent changes of T-wave morphology and U-wave presence. The exploratory endpoint is the ΔΔQTc for ALXN1840 using the concentration-QTc analysis by concentration of total Mo and PUF Mo (as surrogate measures of ALXN1840 PK) in plasma.
For all continuous ECG parameters from each period, Baseline is defined as the average of measured ECG intervals from the 3 predose time points (–45, –30, and –15 minutes before dosing) on Day 1 for the respective period. For T-wave morphology and U-wave presence, Baseline includes findings observed in any replicates from the 3 predose time points (–45, –30, and –15 minutes) on Day 1 in each period.

Pharmacokinetic and Pharmacodynamic Assessments and Endpoints:

Blood samples for PK analysis of total Mo and PUF Mo (as surrogate measures of ALXN1840 PK) will be collected at the following time points: within 1.5 hour before dosing and postdose at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, and 96 hours in each period. Only samples collected predose and following ALXN1840 administration will be analyzed for plasma total Mo and PUF Mo. Samples collected predose and after placebo or moxifloxacin administration will be saved for analysis, if needed.
The following plasma PK parameters will be calculated as endpoints for total Mo and PUF Mo using noncompartmental methods with Phoenix® WinNonlin® (Certara USA Inc., Princeton, New Jersey) Version 8.0 or higher or SAS (SAS Institute Inc., Cary, North Carolina) Version 9.4 or higher, as applicable. Calculations will be based on the actual sampling times recorded during the study.

- Time delay between the time of dosing and time of appearance of Mo concentration in plasma (T_{lag})
- Maximum observed plasma concentration (C_{max})
- Time to reach maximum observed plasma concentration (T_{max})
- Area under the plasma concentration versus time curve (AUC) from time 0 to the last quantifiable concentration (AUC_{0-t})

- AUC from time 0 to 96 hours postdose (AUC_{0-96})
- AUC from time 0 extrapolated to infinity ($AUC_{0-\infty}$)
- Apparent terminal-phase elimination rate constant (λz)
- Terminal elimination half-life ($t_{1/2}$)
- Apparent total clearance from plasma after oral administration of ALXN1840 (CL/F)
-

Pharmacokinetic samples may be used for the quantification of pharmacodynamic (PD) endpoints such as total Cu, PUF Cu, labile-bound Cu, ceruloplasmin, and ceruloplasmin-bound Cu.

Safety:

Safety and tolerability will be assessed by the following endpoints: monitoring and recording of adverse events (AEs), clinical laboratory test results (hematology, serum chemistry, and urinalysis), vital sign measurements, 12-lead safety ECG results, and physical examination findings.

For all safety assessments, the Investigator will determine whether results are clinically significant, which is defined as any variation in a result that has medical relevance and may result in an alteration in medical care (eg, active observation, diagnostic measures, or therapeutic measures). If clinical significance is noted, the result and reason for significance will be documented and an AE will be reported on the AE page of the participant's electronic case report form (eCRF). The Investigator will monitor the participant until the result has reached the reference range or the result at Screening, or until the Investigator determines that follow-up is no longer medically necessary.

Statistical methods:
The statistical hypothesis to be tested for the primary assessment of QT prolongation for the ALXN1840 treatment is:

H_0: $\cup\{\mu_{D(i)} - \mu_{P(i)}\} \geq 10$, i = 1,2,…,12

H_1: $\cap\{\mu_{D(i)} - \mu_{P(i)}\} < 10$, i = 1,2,…,12

where $\mu_{D(i)}$ and $\mu_{P(i)}$ are the least squares (LS) mean of ΔQTc for ALXN1840 and placebo at postdose time point i, respectively.

The by-time point analysis for QTc will be based on a mixed model for repeated measures (MMRM) with change from Baseline QTc (ΔQTc) as the dependent variable; period, sequence, time (ie, postdose time point: categorical), treatment (supratherapeutic dose of ALXN1840, moxifloxacin, and placebo), time-by-treatment interaction as fixed effects, and Baseline QTc and sex as covariates. An unstructured covariance matrix will be specified for the repeated measures at postdose time points for participant within each treatment period. If the model with unstructured covariance matrix fails to converge, other covariance structure such as Toeplitz with heterogeneity (TOEPH), auto-regressive with heterogeneity (ARH), compound symmetry with heterogeneity (CSH), Toeplitz (TOEP), auto-regressive (AR), and compound symmetry (CS) will be considered in decreasing complexity in parameterization. The final model selection will be based on Akaike information criterion (AIC). The degrees of freedom estimates will be determined by the Kenward-Roger method. The model will also include a participant-specific random effect.

Least-squares mean difference and 2-sided 90 % confidence interval (CI) will be calculated for the contrast ALXN1840 versus placebo at each postdose time point, separately. If the upper bound of the 2-sided 90% CI of LS mean ΔΔQTc lies below 10 ms at all 12 postdose time points, ALXN1840 will be concluded not to have a significant effect on QT interval prolongation.

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1. LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

Abbreviation	Term
ΔHR	change from Baseline in heart rate
ΔPR	change from Baseline in PR interval
ΔQRS	change from Baseline in QRS interval
ΔQTc	change from Baseline in the QT interval corrected
ΔQTcF	change from Baseline in the QT interval corrected for heart rate using Fridericia's formula
ΔQTcI	change from Baseline in optimized HR-corrected QT interval
ΔΔHR	placebo-corrected change from Baseline in heart rate
ΔΔPR	placebo-corrected change from Baseline in PR interval
ΔΔQRS	placebo-corrected change from Baseline in QRS interval
ΔΔQTc	placebo-corrected change from Baseline in the QT interval corrected
ΔΔQTcF	placebo-corrected change from Baseline in the QT interval corrected for heart rate using Fridericia's formula
ΔΔQTcI	placebo-corrected change from Baseline in optimized HR-corrected QT interval
ADaM	analysis data model
AE	adverse event
AR	auto-regressive
ARH	auto-regressive with heterogeneity
AUC	area under the plasma concentration versus time curve
AUC_{0-96}	AUC from time 0 to 96 hours postdose
$AUC_{0-\infty}$	AUC from time 0 extrapolated to infinity
AUC_{0-t}	AUC from time 0 to the last quantifiable concentration
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CL/F	apparent total clearance from plasma after oral administration of ALXN1840
C_{max}	maximum observed plasma concentration
CS	compound symmetry
CSH	compound symmetry with heterogeneity
CTCAE	Common Terminology Criteria for Adverse Events
Cu	copper
ECG	electrocardiogram
eCRF	electronic case report form
EOS	end of study
HR	heart rate
ICH	International Council for Harmonisation
IUT	intersection union test
LLOQ	lower limit of quantification
LS	least squares
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation	Term
MMRM	mixed effects model for repeated measures
Mo	molybdenum
NCI	National Cancer Institute
PBC	primary biliary cholangitis
PD	pharmacodynamic
PK	pharmacokinetic(s)
PUF Mo	plasma ultrafiltrate molybdenum
QTc	corrected QT interval
QTcF	QT interval corrected for heart rate using Fridericia's formula
QTcI	optimized HR-corrected QT interval
QTcS	Individualized heart rate corrected QT interval
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SSS	sum of squared slopes
$t_{1/2}$	terminal elimination half-life
T_{max}	time to reach maximum observed plasma concentration
TOEP	toeplitz
TOEPH	toeplitz with heterogeneity
TQT	thorough QT
ULN	upper limit of normal
ULOQ	upper limit of quantification
Vz/F	apparent volume of distribution during the terminal phase
WD	Wilson disease
λz	apparent terminal-phase elimination rate constant

2. INTRODUCTION

This statistical analysis plan (SAP) relates to Alexion Pharmaceuticals Inc. Protocol ALXN1840-HV-107 "A Randomized, Three-Treatment, Three-Period, Six-sequence, Crossover, Placebo- and Active-Controlled, Double-Blind for ALXN1840 (Open-Label for Moxifloxacin) Thorough QT/QTc (TQT) Study to Evaluate the Effect of ALXN1840 on Cardiac Repolarization in Healthy Adult participants", Amendment 1.

ALXN1840 (bis-choline tetrathiomolybdate) is a novel, first-in-class, Cu-protein-binding agent in development for the treatment of Wilson Disease (WD). To follow the International Council for Harmonisation (ICH) E14 guidance on clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs (DHHS, 2005), Study ALXN1840-HV-107 is dedicated to evaluating ALXN1840's potential effect on electrocardiogram parameters, specifically, ALXN1840's effect on cardiac repolarization, as measured by the corrected QT interval.

This document serves as a primary SAP for this study. This document describes the primary, secondary (except PK, PD), and safety analyses planned for this study. PK, PD, and exploratory analysis of ALXN1840 PK concentration on QT/QTc will be covered in a supplemental SAP.

3. STUDY OBJECTIVE(S) AND ENDPOINT(S)

3.1. Study Objective(s)

The primary objective of this study is to evaluate the effect of a supratherapeutic dose of ALXN1840 on the heart rate (HR) -corrected QT interval with the intent to exclude a 10 ms effect.

The secondary objectives of the study are:

- To demonstrate assay sensitivity of the study to detect an effect on the mean QT/QTc interval of approximately 5 ms using moxifloxacin as a positive control.
- To evaluate the safety and tolerability of a single oral supratherapeutic dose of ALXN1840 in healthy participants.
- To assess the PK of ALXN1840 following administration of a single oral supratherapeutic dose in healthy participants.
- To evaluate the effects of a single oral supratherapeutic dose of ALXN1840 on HR, PR and QRS intervals, treatment-emergent T-wave morphology abnormalities, and appearance of U-waves.

The exploratory objective of this study is to evaluate the effects of total Mo and PUF Mo (as a surrogate measure of ALXN1840 PK) concentrations on QT/QTc.

3.2. Study Endpoint(s)

3.2.1. Primary Endpoint

The primary endpoint is placebo-corrected change from Baseline QTc (ΔΔQTc) for ALXN1840 using the by-time point analysis. In the absence of a substantial effect (Section 3.2.2.1.10) on heart rate, the Fridericia method will be used for heart rate correction. If a substantial HR effect is observed, other correction methods such as optimized HR-corrected QT interval (QTcI) may be explored and compared as deemed necessary and appropriate. The method that removes the HR dependence most efficiently will be chosen as the primary correction method and corresponding primary endpoint is ΔΔQTc with this primary correction method for ALXN1840 using the by-time point analysis.

The selected primary correction method will also be used for: 1) Assay sensitivity; and 2) the relationship between total Mo or PUF Mo (as surrogate measures of ALXN1840 PK) concentrations in plasma and ΔΔQTc.

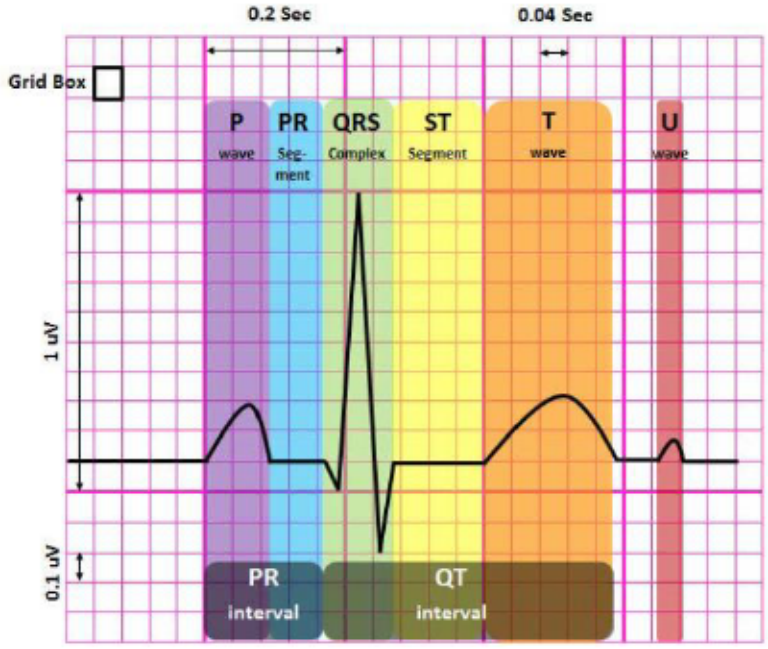
3.2.2. Secondary Endpoints

3.2.2.1. Cardiodynamic Endpoints

- The ΔΔQTc for moxifloxacin;
- The change from Baseline in HR, QTcF, PR, QRS (ΔHR, ΔQTcF, ΔPR and ΔQRS);
- The placebo-corrected change from Baseline HR, PR, and QRS (ΔΔHR, ΔΔPR and ΔΔQRS);
- Categorical outliers for QTcF, HR, PR, and QRS;
- The frequency of treatment-emergent changes of T-wave morphology and U-wave presence.
- If a substantial HR effect (is observed after ALXN1840 administration,
    - The placebo-corrected change from baseline in ΔQTc (ΔΔQTc) using the by-time point analysis (endpoints calculated using correction methods that not used as primary will be considered as secondary)
    - The change from Baseline in QTcI (ΔQTcI)
    - Categorical outliers for QTcI

Figure 1 is a graphic illustration of electrocardiogram wave form, descriptions and algorithms for cardiodynamic endpoints are described in Section 3.2.2.1.1 to Section 3.2.2.1.9

Figure 1: Electrocardiogram Waveform Illustration



3.2.2.1.1. QT Interval

Duration of ventricular activation and recovery; includes the QRS, ST segment, and T-wave.

3.2.2.1.2. QTc Fridericia Correction

The measured QT data will be corrected for heart rate using Fridericia correction method, as per the following formula:

Fridericia's Correction

$$QTc = \frac{QT\ (ms)}{[\frac{RR(ms)}{1000}]^{1/3}}$$

Where RR is the time between 2 consecutive beats/cycles, measured as the time between the peaks of 2 consecutive R waves

3.2.2.1.3. Individualized QT Correction Methods

In case a substantial HR effect (Section 3.2.2.1.10) is observed on-treatment with ALXN1840, drug-free QT/RR data will be collected over a range of HR seen prior to treatment to allow the generation of optimized QT correction method. QTc will be calculated from Day −1 from all evaluable QT/RR pairs in the 24-hour recording (Garnett 2012). These data will be used to obtain the RR interval (HR) and QT data to enable derivation of QTcI as follows.

1. An optimized HR-corrected QT interval (QTcI) will be derived from a broader range of HRs by using all QT/RR data on Day −1 of Treatment Period 1. The QT/RR pairs from each participants will be used for that participant's individual correction coefficient, which will be derived from a linear regression model: $\log(QT) = \log(a) + b \times \log(RR)$. The coefficient of log(RR) for each subject, b_i, will then be used to calculate QTcI for that participant as follows: $QTcI = QT/RR^{bi}$.

2. In addition to QTcI, if needed, individualized HR-corrected QT interval (QTcS) may be calculated from QT/RR data obtained at supine resting time points on Day −1 of Treatment Period 1. Based on QT/RR pairs from all participants, the QTcS correction coefficient will be derived from a linear mixed-effects model: $\log(QT) = \log(a) + b \times \log(RR)$ and sex included as a fixed effect and participant included as a random effect for both intercept and slope. The coefficient of log(RR) for each participant, bi, will then be used to calculate QTcS for each participant as follows: $QTcS = QT/RR^{bi}$.

3.2.2.1.4. PR Interval

PR interval is the time from onset of atrial activation to onset of ventricular activation, it includes P-wave and PR segment.

3.2.2.1.5. QRS Interval

The QRS interval is a series of deflections in an electrocardiogram that represent electrical activity generated by ventricular depolarization prior to contraction of the ventricles, including the Q-wave, R-wave, and S-wave.

3.2.2.1.6. T-wave

T-wave represents ventricular repolarization.

3.2.2.1.7. Changes in T-wave Morphology

T-wave morphology change will be assessed manually, followings are categories of T-wave morphology.

Table 2: Categories for T-Wave Morphology

Category	Description
Normal T-wave	Any positive T-wave not meeting any criterion below
Flat T-wave	T amplitude <1 mm (either positive or negative) including a flat isoelectric line
Notched T-wave (+)	Presence of notch(es) of at least 0.05 mV amplitude on ascending or descending arm of the positive T-wave
Biphasic	T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive/negative and negative/positive and polyphasic T-waves included)
Normal T-wave (-)	T amplitude that is negative, without biphasic T-wave or notches

Category	Description
Notched T-wave (-)	Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave

3.2.2.1.8. U-wave

The U-wave is not observed in all ECGs and when observed, can be either normal or abnormal. U-wave will be assessed manually.

3.2.2.1.9. Heart Rate

The electrical depolarization of cardiac muscle normally precedes contraction of the heart muscle. Under normal circumstances, cardiac contractions result in ejection of blood into the arterial system. The depolarization rate of the cardiac ventricles is referred to as the 'heart rate', which usually equals the number of cardiac contractions (measured as heart beats per minute) and the number of peripheral pulses (measured as the pulse rate).

3.2.2.1.10. Substantial Effect on Heart Rate

Substantial effect on heart rate is defined as the largest LS mean in placebo-corrected change from baseline in ΔHR (ΔΔHR) is greater than 10 bpm in the by-time point analysis.

3.2.2.2. Pharmacokinetic and Pharmacodynamic Assessment and Parameters

The following plasma PK parameters will be calculated as endpoints for total Mo and PUF Mo (as a surrogate measure of ALXN1840) using noncompartmental methods with Phoenix® WinNonlin® (Certara USA Inc., Princeton, New Jersey) Version 8.0 or higher or SAS Version 9.4 or higher (SAS Institute Inc., Cary, North Carolina), as applicable. Calculations will be based on the actual sampling times elapsed from the reference dosing time in the period as recorded during the study.

- Time delay between the time of dosing and time of appearance of Mo concentration in plasma (T_{lag})
- Time (observed time point) of last quantifiable concentration (T_{last})
- Maximum observed concentration in plasma (C_{max})
- Time to reach maximum observed concentration in plasma (T_{max})
- Area under the concentration in plasma versus time curve (AUC) from time 0 to the last quantifiable concentration (AUC_{0-t})
- AUC from time 0 to 96 hours postdose (AUC_{0-96})
- AUC from time 0 extrapolated to infinity ($AUC_{0-\infty}$)
- Apparent terminal-phase elimination rate constant (λz)

- Terminal elimination half-life ($t_{1/2}$)
- Apparent total clearance from plasma after oral administration of ALXN1840 (CL/F)
- Apparent volume of distribution during the terminal phase (Vz/F)

  Additional PK parameters may be calculated if deemed appropriate.

Pharmacokinetic samples may be used for the quantification of PD endpoints such as total Cu, PUF Cu, labile-bound Cu, ceruloplasmin, and ceruloplasmin-bound Cu.

3.2.2.3. Safety Endpoints

- Adverse Events
- Clinical laboratory test results (hematology, serum chemistry, and urinalysis)
- Vital signs measurements
- 12-lead safety ECG results
- Physical examination findings

3.2.3. Exploratory Endpoints

The exploratory endpoint is the relationship between total Mo or PUF Mo (as surrogate measures of ALXN1840 PK) concentrations in plasma and ΔΔQTc.

3.3. Statistical Hypotheses

3.3.1. Primary Hypothesis

The statistical hypothesis to be tested for the primary assessment of QT prolongation for the ALXN1840 treatment is:

H_0: $\cup \{\mu_{D(i)} - \mu_{P(i)}\} \geq 10$, $i = 1,2,\ldots,12$

H_1: $\cap \{\mu_{D(i)} - \mu_{P(i)}\} < 10$, $i = 1,2,\ldots,12$

where $\mu_{D(i)}$ and $\mu_{P(i)}$ are the LS mean of ΔQTc for ALXN1840 and placebo at postdose time point i (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours postdose), respectively.

If the upper bound of the 2-sided 90% CI of LS mean ΔΔQTc lies below 10 ms at all 12 postdose time points, ALXN1840 will be concluded not to have a significant effect on QT interval prolongation.

3.3.2. Secondary Hypothesis

The secondary hypothesis to be tested for the secondary assessment of QT prolongation for the moxifloxacin

H_0: $\mu_{M(i)} - \mu_{P(i)} \leq 5$, i = 1, 2, 3

H_1: $\mu_{M(i)} - \mu_{P(i)} > 5$, i = 1, 2, 3

where $\mu_{M(i)}$ and $\mu_{P(i)}$ are the LS mean of ΔQTc for moxifloxacin and placebo at postdose time point i (1, 2, and 3 hours postdose), respectively. Multiplicity will be controlled by using a Hochberg procedure (Hochberg, 1988) with adjusted 1-sided significance levels of 5%, 2.5%, and 1.67% for statistical testing, respectively, of the 3 p-values ranked from largest to smallest. The largest p-value will be evaluated using a 5% significance testing level and the smallest will be evaluated using a 1.67% significance testing level. Testing will start with largest p-value, and continue sequentially through the ranked p-values, until the first significant value is found at which point all smaller p-values are considered significant. If at least 1 test is significant at the specified level, assay sensitivity will be concluded.

4. STUDY DESIGN

This is a randomized, 3-treatment, 3-period, 6-sequence, crossover, placebo- and active-controlled, double-blind for ALXN1840, open-label for moxifloxacin study in healthy adult participants. Moxifloxacin will be used as the active control.

Approximately 54 participants will be randomized to ensure there will be 45 evaluable participants with data from all treatment periods. To ensure sequences are balanced within sex, participant randomization will be stratified by sex. Study intervention will be given in randomized sequences: ABC, ACB, BAC, BCA, CAB, and CBA, with each intervention administered with 240 mL of water following an overnight fast of at least 10 hours. No food will be allowed for at least 4 hours postdose. Water can be allowed as desired except for 1 hour before and after drug administration:

Cardiodynamic assessment will be performed for approximately 25 hours on Day –1 of Treatment Period 1 and Day 1 to Day 2 of each treatment period. Blood samples for PK will be collected predose and at specified times up to 96 hours after the Day 1 dose in each treatment period.

Twelve-lead electrocardiograms will be extracted from a continuous (Holter) recording by the central laboratory. To support high quality data for extraction, 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. Blood for plasma PK will be collected as close as possible to nominal time after completion of ECG extraction with actual times for blood sample collections recorded.

Participants will be domiciled in the clinic for 7 days in Treatment Period 1 and for 6 days in Treatment Periods 2 and 3, starting on Day –2 for Treatment Period 1 and on the day before dosing (Day –1) for Treatment Periods 2 and 3, until safety procedures have been completed and reviewed on Day 5. There will be a washout of at least 14 days between dose administration in each period.

All participants (including participants who terminate the study early) will return to the study site 14 days (±2 days) after the last administration of study intervention for follow-up procedures and to determine if any AE has occurred since the last study visit.

Duration of participation for each participant from the Screening Visit to the End-of-Study (EOS) Visit will be approximately 70 days. This includes up to 29 days for the Screening Period, study intervention administration on Day 1 of each treatment period, a minimum of a 14-day washout between study intervention administration in each treatment period, and 14 days (±2 days) for the EOS Visit.

4.1. Definition of Study Drugs

Treatment A: A single oral dose of ALXN1840 120 mg administered as 15-mg enteric-coated tablets (supratherapeutic dose)

Treatment B: A single oral dose of enteric-coated placebo tablets matching ALXN1840

Treatment C: A single oral dose of moxifloxacin 400 mg

4.2. Sample Size Considerations

4.2.1. Sample Size Justifications

A sample size of approximately 54 participants was chosen to obtain 45 evaluable participants to complete the study. Assuming a 1-sided 5% significance level and a within-participant SD of 8 ms for ΔQTc for all treatment groups and a true mean difference of 3 ms in ΔQTc between ALXN1840 and placebo, based on the calculation of the sample size for a TQT study (Zhang, 2008), a sample size of 45 evaluable participants will provide a power of 90% to demonstrate that the upper bound of all the 2-sided 90% CIs on ΔΔQTc will fall below 10 ms for up to 12 postdose time points. To account for a drop-out rate of approximately 16%, 54 participants will be enrolled.

Based on the calculation of the sample size for a TQT study (Zhang, 2008), as the test is performed at 3 time points separately (1, 2, and 3 hours), a 1-sided 5% significance level (with adjusted 1-sided significance levels of 5%, 2.5%, and 1.67%) is used along with a within-participant SD of 8 ms for ΔQTc and a true effect of moxifloxacin of 10 ms, a sample size of 45 evaluable participants will provide > 98% power to demonstrate assay sensitivity of excluding a mean difference of 5 ms in ΔQTc between moxifloxacin and placebo groups.

4.3. Randomization

A randomization schedule will be generated, and randomization numbers will be assigned before the first dose of study intervention is administered on Day 1 of Period 1.

After meeting all inclusion and none of the exclusion criteria, participants will be randomized to one of the 6 pre-specified treatment sequence. To ensure sequences are balanced within sex, randomization will be stratified by sex.

4.4. Clinical Assessments

Phase	Screening	Same Schedule for Treatment Periods 1, 2, and 3																				EOS
		Check-in (Day -2 for Period 1; Day -1 for Periods 2 and 3																				
Day	–29 to –2	–2	–1	1														2	3	4	5	15 ±2
Procedure[a] Hours	–	–	–	-0.75	-0.5	-0.25	0	0.5	1	2	3	4	5	6	7	8	10	12	24	48	72	96	–
Admission to clinic[b]		X	X																				
Discharge from clinic[c]																						X	
Outpatient visit[d]																							X
Informed consent	X																						
Inclusion/exclusion criteria	X	X	X																				
Medical history Error! Reference source not found.	X																						
Demographics	X																						
Serology	X																						
Serum FSH[f]	X																						
Serum copper and ceruloplasmin	X																						
Height, weight, and BMI[g]	X	X	X																				X
Physical examination[h]	X	X	X																				X
Vital sign measurements[i]	X	X	X	X						X								X	X	X	X	X
12-lead safety ECG[j]	X	X	X	X								X		X		X		X	X			X	X
Clinical laboratory testing	X	X	X															X				X	X
Urinalysis	X	X	X																				X
Drug/alcohol/cotinine screen	X	X	X																				
Serum pregnancy test[k]	X	X	X																				X
Randomization (Period 1 only)				X																			
Study intervention administration[l]							X																
12-lead Holter ECG[m]			X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X				
PK blood sample[n]				X				X	X	X	X	X	X	X	X	X	X	X	X	X		X	
Fasting period			X	X	X	X	X	X	X	X	X	X											
Non-fasting period													X	X	X	X	X	X	X	X	X	X	X
Adverse events	◄									X													►

Nonpharmacologic therapies and procedures	◄——— X ———►
Prior/concomitant medications	◄——— X ———►

Abbreviations: AEs, adverse events; BMI, body mass index; ECG, electrocardiogram; EOS, end of study; FSH, follicle-stimulating hormone; PK, pharmacokinetic.
Notes:

[a] When procedures overlap or occur at the same time point, all blood draws should follow vital signs or ECGs, and PK sampling should be timed to occur last and as close to the scheduled time window as possible.

[b] Participants will be asked to arrive at the study site on Day –2 of Treatment Period 1 and on the day before the start of dosing (Day –1) of Treatment Periods 2 and 3. The assessments scheduled for Day –2 of Treatment Period 1 will not be repeated on Day –1 of Treatment Period 1.

[c] Discharge from the study site will occur after the 96-hour PK samples and after completion and review by the Investigator of all 96-hour safety assessments (including safety laboratory test results) on Day 5 of each study period. There will be a washout of at least 14 days between dose administration in each period.

[d] The EOS Visit will occur 14 days (±2 days) after the last dose of study intervention in Period 3 or upon early discontinuation.

[e] A full medical history will be performed at Screening only. Any subsequent changes to health status will be assessed at each Check-in.

[f] Females only.

[g] Height and weight will be measured and BMI calculated at Screening only. Only weight will be measured at Check-in and EOS.

[h] A full physical examination will be performed at Screening. A brief physical examination will be performed at Check-in and EOS.

[i] Predose vital signs on Day 1 may be obtained up to 1.5 hours before dosing.

[j] Predose ECG on Day 1 may be obtained up to 1.5 hours before dosing.

[k] Women only.

[l] The time of study intervention (ALXN1840, placebo, or moxifloxacin) dosing will be called "0" hour in each period.

[m] Continuous 12-lead Holter ECG will be performed on Day -1 of Period 1 only to record the baseline ECG, which will allow extraction of ECG recordings that match the time points of Day 1 of Periods 1, 2 and 3. On Day -1, participants shall follow the Day 1 supine positioning schedule. Participants shall assume supine positioning starting at least 15 minutes before the Day 1 timepoints for ECG extraction and continue to remain in the supine position until 5 minutes after the timepoint. The -0.25 hour pre-dose ECG time point will be used as the 24-hours ECG time point of Day -1, Period 1.

[n] Predose samples on Day 1 may be obtained up to 1.5 hours before dosing.

5. PLANNED ANALYSES

5.1. Interim Analyses

No interim analysis is planned for this study.

5.2. Final Analysis

The final analysis will be performed following the database lock after the last participant has completed the final follow-up assessment.

5.3. Changes from Analysis Specified in the Protocol

The original correction method proposed for primary endpoint is placebo-corrected change from Baseline QTcF (ΔΔQTcF) for ALXN1840 using the by-time point analysis.

Which correction method to be used as primary correction will be based on whether there is a substantial HR effect (Section 3.2.2.1.10) on-treatment with ALXN1840, other correction method such as optimized HR-corrected QT interval (QTcI) may be used as appropriate in the presence of substantial HR effect.

The selected primary correction method will also be used for: 1) Assay sensitivity; and 2) the relationship between total Mo or PUF Mo (as surrogate measures of ALXN1840 PK) concentrations in plasma and ΔΔQTc.

5.3.1. Primary Endpoint

The primary endpoint is placebo-corrected change from Baseline in QTc (ΔΔQTc) for ALXN1840 using the by-time point analysis.

5.3.2. Secondary endpoints

- The ΔΔQTc for moxifloxacin

If a substantial HR effect is observed after ALXN1840 administration,

- The placebo-corrected change from baseline in ΔQTc (ΔΔQTc) using the by-time point analysis (Endpoints calculated using correction methods that not used as primary will be considered as secondary)
- The change from Baseline in QTcI (ΔQTcI)
- Categorical outliers for QTcI

5.3.3. Exploratory endpoint

The exploratory endpoint is the relationship between total Mo or PUF Mo (as surrogate measures of ALXN1840 PK) concentrations in plasma and ΔΔQTc.

5.3.4. PK endpoint

The following endpoint is added

- Time (observed time point) of last quantifiable concentration (T_{last})

6. DATA HANDLING, PRESENTATION CONVENTIONS, AND DEFINITION

6.1. Data Management

Study data identified in the schedule for time and events (Section 4.4) will be collected, and source verified, on the electronic data capture tool RAVE. Clinical laboratory testing data, ECG data, and PK data are not collected in the RAVE and are provided from external laboratories. Data storage, data transfer and cleaning process will be covered in the data management plan.

All study data will be formulated into regulatory compliant data sets. Data will be mapped to the Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model (SDTM) and serve as the source data from the trial. All study analyses will be completed using analysis data sets that are derived from the SDTM and follow the CDISC Analysis Data Model (ADaM) architecture.

Data manipulation and analyses will be performed primarily using SAS version 9.4 or higher (SAS Institute Inc., Cary, North Carolina).

6.2. Data Presentation Conventions

Continuous variables (e.g. age) are summarized using descriptive statistics (the number of participants with available data, the mean, standard deviation (SD), median and minimum and maximum). Categorical variables (e.g. race) are summarized using counts and percentages.

The following conventions are applied to all data 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.
- For categorical variables, the number and percentage of responses are presented in the form XX (XX.X%) where the percentage is in the parentheses.
- Date variables are formatted as DDMMMYYYY for presentation. Time is formatted in military time as HH:MM for presentation.
- Wherever possible, data will be decimal aligned.
- P-values, if applicable, will be presented to 4 decimal places. If the p-value is less than 0.0001 then it will be presented as <0.0001. If the rounded result is a value of 1.000, it will be displayed as >0.9999.

6.3. Analysis Sets

6.3.1. Screened Set

The Screened Set will include all participants who signed informed consent form.

6.3.2. Enrolled Set

The Enrolled Set will include all participants who are randomized.

6.3.3. Safety Set

The Safety Set will include all participants who receive at least 1 dose of study intervention (ALXN1840, moxifloxacin, or placebo) and for whom any safety data are available.

6.3.4. QT/QTc Set

The QT/QTc Set will include all participants in the Safety Set with measurements at Baseline as well as on-treatment with at least 1 postdose time point with a valid ΔQTc value. The QT/QTc Set will be used for the by-time point, assay sensitivity, and categorical analyses of the cardiodynamic ECG parameters.

6.3.5. PK Set

The PK Set will include all participants who receive at least 1 dose of ALXN1840 and have evaluable PK data for total Mo and/or PUF Mo (as surrogate measures of ALXN1840 PK) in plasma. The PK Set will be used for PK analysis.

6.3.6. PD Set

The PD Set will include all participants who receive at least 1 dose of ALXN1840 and have evaluable PD data for total Cu and/or PUF Cu in plasma. The PD Set will be used for PD analysis.

6.3.7. PK/QTc Set

The PK/QTc Set will include all participants who are in both the QT/QTc and PK Sets with at least 1 pair of postdose PK and QTc data from the same time point as well as participants in the QT/QTc Set who received placebo. The PK/QTc Set will be used for the exploratory concentration-QTc analysis.

6.4. Baseline Definition

Unless otherwise noted, Baseline assessments are defined as the last non-missing result prior to administration of the first dose of study intervention (e.g., prior first dose in the first treatment period).

6.5. Baseline ECG Parameters, T-wave, U-wave

6.5.1. Predose Baseline

ECG parameters predose Baseline (HR, PR, QRS, QT, and QTc) in each period are defined as the average of the measured ECG intervals from the 3 predose time points (−45, −30, and −15 minutes before dosing) on Day 1 in each treatment period.

For T-wave morphology abnormality and U-wave presence in each period, predose Baseline will be based on findings observed in the any replicates from the 3 predose time points (−45, −30, and −15 minutes) on Day 1 in each treatment period.

6.6. Derived and Transformed data

6.6.1. Baseline Age

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

Age (year) = FLOOR((reference date – date of birth)/365.25)

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

In cases where only the month and year are provided for a date, the day for the date will be imputed as 15. Missing month will be imputed as June. In cases where the day is observed but the month is missing, the date will be imputed as June 15.

6.6.2. Study Day

If the date of interest occurs on or after the first dose/randomization date, then study day will be calculated as (date of interest – date of first dose/randomization) + 1. If the date of interest occurs prior to the first dose/randomization date, then study day will be calculated as (date of interest – date of first dose/randomization). There is no study day 0.

6.6.3. Change from Baseline

Change from Baseline is calculated as (Post-baseline result – Baseline result). If either the Baseline or the Post-baseline result is missing, the change from Baseline is set to missing as well.

6.6.4. Change from Predose Baseline ECG Parameters

For cardiodynamic analyses, change from predose Baseline ECG parameters (ΔQTc, ΔHR, ΔPR, ΔQRS) is calculated as (Post-baseline ECG parameter at a specific timepoint minus predose Baseline ECG parameter) for each treatment period.

6.6.5. Placebo-corrected Change from Baseline ECG Parameters

In the primary and secondary by-time point analysis, placebo-corrected change from baseline ECG measures and 90% CI (e.g. ΔΔQTc, ΔΔHR, ΔΔPR, ΔΔQRS) will be estimated from statistical model at each time point.

7. GENERAL CONSIDERATIONS FOR DATA ANALYSES

7.1. Multicenter Studies

N/A

7.2. Other Strata and Covariates

This is a clinical pharmacology study in a healthy adult population. It is not expected that any of the variety of Baseline demographic parameters would introduce a large QT/QTc response to ALXN1840 in certain sub-population defined as age, race, etc.

7.3. Examination of Subgroups

For exploratory purposes, primary endpoint will be evaluated by sex.

7.4. Multiple Comparisons and Multiplicity

The primary analysis will be based on by-time point analysis to evaluate the effect of ALXN1840 on the ΔΔQTc at each postdosing time point ("by-time point" analysis) using the intersection union test (IUT) procedure at one-sided 0.05 level. The IUT procedure is conservative in that the type I error rate might be smaller than the intended 0.05 level. There is no multiplicity adjustment for the primary analysis.

The secondary hypothesis for assay sensitivity will be based on by-time point analysis to evaluate the effect of moxifloxacin on the ΔΔQTc at the 3 predefined time points (1, 2, and 3 hours postdose). Multiplicity will be controlled by using a Hochberg procedure, with adjusted 1-sided significance levels of 5%, 2.5%, and 1.67% for statistical testing, respectively, of the 3 p-values ranked from largest to smallest. The largest p-value will be evaluated using a 5% significance testing level and the smallest will be evaluated using a 1.67% significance testing level. Testing will start with largest p-value, and continue sequentially through the ranked p-values, until the first significant value is found at which point all smaller p-values are considered significant. If at least 1 test is significant at the specified level, assay sensitivity will be concluded.

7.5. Data handling conventions

7.5.1. Premature Withdrawal and Missing Data

Every effort will be made to collect all data. However, despite best efforts, missing or incomplete information may occur. All missing or partial data will be presented in the subject data listing, as they are recorded on the eCRF.

In the event of missing dose, the data collected in the associated period will be excluded from analyses.

Participants lost to follow-up or premature withdrawn will be included in statistical presentations up to the point of their last evaluation. Missing data will not be imputed. Analyses will be based on observed values.

7.5.2. Missing Dates

Unless otherwise specified, missing date will not be imputed. Partial start dates/times will be queried.

For adverse events, if information is not available to reliably allocate AE to a period, the allocation will be agreed at the data review meeting before database lock. If there is any doubt about ALXN1840 treatment emergence, AEs will be classified as ALXN1840 treatment-emergent.

For concomitant medication, if medication dates or times are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant.

8. STATISTICAL ANALYSIS

8.1. Disposition of Participants

The number of participants who enrolled in the study will be presented. The number and percentages of participants included in each analysis set will be summarized by treatment sequence and overall.

The number and percentage of participants who completed, or prematurely discontinued from the study, along with reason for discontinuation will be described by randomized treatment sequence and overall.

Prematurely discontinued participants will be presented in a listing. A list of screening failure will also be provided.

8.2. Protocol Deviations

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

8.3. Demographic and Baseline Safety Characteristics

Individual subject demographics (including age, sex, race and ethnicity), body measurement data (height, body weight and body mass index) will be listed and summarized for the Safety Set by each treatment sequence and overall. If the number of participants in any of the QT/QTc, PK, PD, and PK/QTc sets are different from the Safety Set by more than 5%, separate demographic and baseline characteristic tables will be produced for that set.

Data collected for virus serology, urine drug screen, serum copper, ceruloplasmin and serum pregnancy test (for women of childbearing potential) will be provided in listing.

8.4. Medical History

Medical history data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary Version 22.0 (or higher). Medical and surgical history data will be listed individually and summarized by treatment sequence and overall for the Safety Set.

8.5. Prior and Concomitant Medication

The World Health Organization Drug Dictionary version from March 2018 or later will be used to code the medications.

Prior medications are defined as therapies ended before the date and time of first study intervention administration.

Concomitant medications are defined as those either with start date and time on or after the date and time of first study intervention administration, or those with start date and time prior to the first study intervention drug administration and continuing at the time of first study intervention administration.

Prior and concomitant medications will be summarized for the Safety Set by sequence group and overall. The number and percentage of participants receiving any concomitant medication will be summarized, as well as the number and percentage receiving any concomitant medication by ATC drug class and generic drug name. Participants reporting use of more than one medication at each level of summarization (any medication received, ATC class, and generic drug name) will be counted only once. ATC class terms will be displayed by descending order of incidence, as well as generic drug names within each ATC class.

Prior and concomitant medications will also be presented in individual listing.

8.6. Treatment Compliance

As per protocol, study intervention dosing will occur with the participant being in a fasted state. The date and time of study intervention dosing, and nutritional state (fasting/non-fasting) will be provided in listing, for participants who missed study intervention, the reason for the missed dose will be provided in the listing for the Safety Set.

8.7. Cardiodynamic Analyses

Cardiodynamic analyses will be based on the QT/QTc Set. For cardiodynamic analyses, Baseline refers to predose Baseline.

ECG parameters (QTc, HR, PR, QRS) and change from Baseline (ΔQTc, ΔHR, ΔPR, ΔQRS) at all time points will be summarized using descriptive statistics by treatment and time. Change from Baseline in ECG parameters will also be presented by treatment and time using figures.

Model based least square means of ΔΔQTc and 90% CIs will be plotted against measured time points for ALXN1840 and Moxifloxacin individually as well as overlaying estimates of these two treatments in one figure.

8.7.1. Evaluation of QT/RR Correction Methods

In case a substantial HR effect is observed on-treatment with ALXN1840, QTcI will be derived and compared to QTcF using evaluation method that described below.

The relationship between QTc (QTcF and, if derived, QTcI [optimized]) and RR interval will be investigated using on-treatment data (ALXN1840, moxifloxacin, and placebo) by a linear regression model: $QTc = c + d \times RR$. Mean QTc and RR values from all nominal time points (including predose) will be used. The RR coefficient for each subject, d_i, will then be used to calculate the average sum of squared slopes (SSS) for each of the different QT/RR correction methods. The correction method that results in the average on-treatment slope closest to 0 (the smallest average SSS, as described by Tornøe (Tornøe CW 2011) for ALXN1840 and placebo

will be deemed the most appropriate HR correction method. If different methods show lowest SSS on placebo as compared to ALXN1840, priority in the choice will be given to the placebo results. In addition, a scatter plot and quantile plot of QTc (QTcF, and QTcI) and RR intervals by treatment with regression line and a linear mixed-effects line (90% CI), respectively, will also be given.

8.7.2. Primary Analysis(es)

The primary analysis will be based on by-time point analysis to evaluate the effect of ALXN1840 on the ΔΔQTc at each postdosing time point ("by-time point" analysis) using the intersection union test (IUT).

The statistical hypothesis to be tested for the primary assessment of QT prolongation for the supratherapeutic dose of the ALXN1840 treatment is:

H_0: $\cup\{\mu_{D(i)} - \mu_{P(i)}\} \geq 10$, $i = 1,2,\ldots,12$

H_1: $\cap\{\mu_{D(i)} - \mu_{P(i)}\} < 10$, $i = 1,2,\ldots,12$

where $\mu_{D(i)}$ and $\mu_{P(i)}$ are the least squares (LS) mean of ΔQTc for ALXN1840 and placebo at postdose time point i, respectively.

The by-time point analysis for QTc will be based on a mixed model for repeated measures with change from Baseline QTc as the dependent variable, period, sequence, time (ie, postdose time point: categorical), treatment (supratherapeutic dose of ALXN1840, moxifloxacin, and placebo), time-by-treatment interaction as fixed effects; and Baseline QTc and sex as covariates. An unstructured covariance matrix will be specified for the repeated measures at postdose time points for participant within treatment period. If the model with unstructured covariance matrix fails to converge, other covariance structure such as Toeplitz with heterogeneity (TOEPH), auto-regressive with heterogeneity (ARH), compound symmetry with heterogeneity (CSH), TOEP, AR, and CS will be considered in decreasing complexity in parameterization. The final model selection will be based on Akaike information criterion (AIC). The degrees of freedom estimates will be determined by the Kenward-Roger method. The model will also include a participant-specific random effect.

Least-squares mean difference and 2-sided 90 % CI will be calculated for the contrast ALXN1840 versus placebo at each postdose time point, separately. If the upper bound of the 2-sided 90% CI of LS mean ΔΔQTc lies below 10 ms at all 12 postdose time points, ALXN1840 will be concluded not to have a significant effect on QT interval prolongation.

Sample code for primary analysis:

```
PROC MIXED DATA=ECG METHOD=REML;
CLASS SUBJID TREAT TIME PERIOD SEQUENCE SEX;
MODEL QTc=BASE TREAT TIME TREAT*TIME PERIOD SEQUENCE SEX/DDFM=KR;
RANDOM INTERCEPT / SUBJECT =SUBJID TYPE=UN;
REPEATED TIME / SUBJECT = PERIOD*SUBJID TYPE=UN;
LSMEANS TREAT*TIME/CL DIFF ALPHA=0.1;
```

```
* Example for obtaining p-value at a specific time point;
LSMESTIMATE TREAT*TIME 'ALXN1840 VS PLACEBO AT POST-DOSE 24 HRS'
            1 0 0 0 0 0 0 0 0 0 0 0 0
            0 0 0 0 0 0 0 0 0 0 0 0 0
            -1 0 0 0 0 0 0 0 0 0 0 0 0/TESTVALUE=10 ALPHA=0.05 LOWERTAILED CL;
RUN;
```

Where ECG = QT/QTc set, SUBJID = subject number, TREAT = treatment (supratherapeutic dose of ALXN1840, moxifloxacin, and placebo), TIME = nominal post-dose time point, BASE = baseline QTc

8.7.3. Assay Sensitivity Analysis(es)

Assay sensitivity will also be evaluated using by-time point analysis of the effect on ΔΔQTc of moxifloxacin using the same model as for the primary analysis. The analysis to show assay sensitivity will be based on the change from Baseline in QTc of moxifloxacin. For the 3 predefined time points (1, 2, and 3 hours postdose), the contrast in treatment ΔΔQTc = moxifloxacin versus placebo will be tested against the 1-sided null hypothesis ΔΔQTc ≤5 ms on the 5% level. Multiplicity will be controlled by using a Hochberg procedure (Hochberg 1998) with adjusted 1-sided significance levels of 5%, 2.5%, and 1.67% for statistical testing, respectively, of the 3 p-values ranked from largest to smallest. The largest p-value will be evaluated using a 5% significance testing level and the smallest will be evaluated using a 1.67% significance testing level. Testing will start with largest p-value, and continue sequentially through the ranked p-values, until the first significant value is found at which point all smaller p-values are considered significant.

If after this procedure the LS mean of ΔΔQTc is significantly larger than 5 ms for at least 1 time point of these 3 time points, assay sensitivity will be considered to have been demonstrated. In addition, 2-sided 90% CIs will be obtained for the contrast at all time points and used in the figures.

Sample code for primary analysis:

```
PROC MIXED DATA=ECG METHOD=REML;
CLASS SUBJID TREAT TIME PERIOD SEQUENCE SEX;
MODEL QTc=BASE TREAT TIME TREAT*TIME PERIOD SEQUENCE SEX/DDFM=KR;
RANDOM INTERCEPT / SUBJECT =SUBJID TYPE=UN;
REPEATED TIME / SUBJECT = PERIOD*SUBJID TYPE=UN;
LSMEANS TREAT*TIME/CL DIFF ALPHA=0.1;

*Example for obtaining p-value at a specific time point;
LSMESTIMATE TREAT*TIME 'MOXI VS PLACEBO AT POST-DOSE 1 HR'
            0 0 0 0 0 0 0 0 0 0 0 0 0
            0 0 0 0 0 0 0 0 0 0 0 1 0
            0 0 0 0 0 0 0 0 0 0 0 -1 0/TESTVALUE=5 ALPHA=0.05 UPPERTAILED CL;
RUN;
```

8.7.4. Other Cardiodynamic Analysis(es)

8.7.4.1. Other Continuous Cardiodynamic Endpoints

For HR, PR, QRS, and QTc correction method not selected as primary if there is substantial HR effect, by-time point analysis will be used to evaluate the effect of ALXN1840 on the ΔΔHR, ΔΔPR, ΔΔQRS and ΔΔQTc at each postdosing time point. Similarly, MMRM model will be used to model change from Baseline in ΔHR, ΔPR, ΔQRS, and ΔQTc. Fixed effects will include period, sequence, time (ie, postdose time point: categorical), treatment (supratherapeutic dose of ALXN1840, and placebo), time-by-treatment interaction and corresponding predose Baseline measures of that ECG parameter and sex as covariates. The LS mean, SE, and 2-sided 90% CI from the statistical modeling for both change from Baseline and placebo-corrected change from Baseline values will be presented in the tables and graphically displayed.

8.7.4.2. Categorical Endpoints

An analysis of categorical outliers (including all replicates at all time points) will be performed for changes in HR, PR, QRS, QTc, T-wave morphology, and U-wave presence. The results for categorical outliers will be summarized in frequency tables with counts (percentages) for both number of participants and number of time points by treatment. The frequency of participants with an increase from Baseline in ECG measures will be summarized for each treatment according to the following categories:

- QTcF values >450 and ≤480 ms, >480 and ≤500 ms, or >500 ms;
- Increase in QTcF from predose Baseline >30 and ≤60 ms, or >60 ms;
- QTcI values >450 and ≤480 ms, >480 and ≤500 ms, or >500 ms if there is substantial HR effect;
- Increase in QTcI from predose Baseline > 30 and ≤ 60 ms, or >60 ms if there is substantial HR effect;
- Increase in PR from predose Baseline >25% to a PR >200 ms;
- Increase in QRS from predose Baseline >25% to a QRS >120 ms;
- Decrease in HR from predose Baseline >25% to a HR <50 bpm;
- Increase in HR from predose Baseline >25% to a HR >100 bpm;

Individual participant ECG data will be listed. All incidences of ECG measures that fall into above categories will be flagged in the individual data listing.

For T-wave morphology, and U-wave presence, summarize the number (%) of participants with at least: 1) one treatment-emergent T-wave change; 2) one treatment-emergent U-wave abnormality, with data summarized by treatment and overall. Individual participant data on T-wave morphology

and abnormal U-wave presence will be listed, treatment emergent T-wave morphology abnormal change and abnormal U-wave will be flagged.

8.8. Pharmacokinetic and Pharmacodynamic Analyses

Details of the PK and PD analyses will be described in a separate PK/PD, and QT/QTc SAP.

8.9. Pharmacokinetic Concentration/QTc Analysis (Exploratory Analysis)

Details of the PK concentration/QTc analysis will be described in a separate PK/PD, and QT/QTc SAP.

8.10. Safety Analyses

Safety Analyses will be based on the Safety Set.

8.10.1. Adverse Events

Treatment-emergent adverse events (TEAEs) are defined as those AEs with onset after the first dose of study intervention or existing events that worsened in severity after the first dose of study intervention.

Adverse events will be coded by preferred term and system organ class using MedDRA version 22.0 or higher and summarized by primary SOC and PT. Adverse event severity will be evaluated using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (published 27 Nov 2017) (NCI, 2017).

A TEAE will be classified to a specific Period if the AE start date/time is on or after the date and time of treatment administration of that Period and before the start date/time of the next Period.

All TEAE data will be presented in a data listing. Treatment-emergent SAE and AEs leading to early discontinuation will also be presented in the separate data listings.

8.10.2. Overall Summary of Adverse Events

An overall summary of TEAEs will be presented, number of events, number and percentage (%) of participants experiencing the event will be summarized by treatment and overall. The summary will include following categories:

- TEAEs
  - Related
  - Non-related
  - Leading to early discontinuation

  - Leading to death

- Treatment-emergent SAEs
  - Related
  - Non-related
  - Leading to early discontinuation
  - Leading to death
- TEAEs by toxicity grade
- Treatment-emergent SAEs by toxicity grade

8.10.3. AEs by System Organ Class and Preferred Term

The number of TEAEs and the number and percentage of participants with events will be presented by system organ class and preferred term. Participants are counted once in each system organ class and preferred term. Data will be summarized by treatment and overall. System organ classes will be listed in descending frequency as well as preferred terms within each system organ class.

Treatment-emergent SAEs, treatment-emergent non-serious AEs, related TEAEs, related treatment-emergent SAEs will be summarized using the same approach.

8.10.4. AEs by Preferred Term

The number of TEAEs and the number and percentage of participants with events will be presented by preferred term in column for overall. Participants are counted once in each preferred term. Data will be summarized by treatment and overall.

8.10.5. AEs by System Organ Class, Preferred Term and Severity

The number of TEAEs and the number and percentage of participants with events will be presented by system organ class, preferred term and severity. If a participant has more than one occurrence of an AE, the highest severity reported will be used. If severity is missing, the AE will be assumed to be severe.

8.11. Clinical Laboratory Evaluations

Clinical laboratory parameters (including blood chemistry, haematology, serum chemistry, and urinalysis) will be graded according to the CTCAE. Absolute (observed) values and changes from Baseline (continuous variables) will be summarized for each parameter at the scheduled time point by treatment. The last lab value will be used for summary analysis if repeated measurements are made at any time point.

For descriptive statistics of clinical laboratory data values that are below the lower limit of quantification (LLOQ) will be treated as LLOQ and those above the upper limit of quantification (ULOQ) will be treated as ULOQ. Missing concentrations will be excluded from the calculations.

Contingency tables will be presented for each laboratory parameter to summarize the shift from the Baseline category to the worst Post-baseline measurement by treatment.

8.12. Safety Electrocardiogram

The Baseline for safety ECG measures refers to predose Baseline before each treatment period.

12-lead safety ECG measures including HR, PR, QTc, QRS and change from baseline in those parameters will be analyzed using descriptive summary statistics by treatment group. For ECG parameters, the average of all the replicates at measured time points will be used for statistics analyses.

Listings of 12-lead safety ECG with interpretation, morphology analysis and flag for clinically significant will be provided.

8.13. Vital Signs

Vital sign data including systolic and diastolic blood pressure, pulse rate, respiratory rate, and body temperature will be listed for each individual participant.

Summary statistics of observed values and changes from Baseline will be presented for each parameter at scheduled time point by treatment.

8.14. Physical Examination

Physical examination data will be listed individually, and abnormal findings will be flagged.

9. REFERENCES

Department of Health and Human Services (DHHS), Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research (US). Guidance for Industry: E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs. October 2005 [cited 02 Dec 2019] [20 screens]. Available from: https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM073153.pdf.

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PPD PPD PPD 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: 03-Sep-2020 | 08:28 Viewed: 03-Sep-2020 | 08:28 Signed: 03-Sep-2020 | 08:29
Electronic Record and Signature Disclosure: Not Offered via DocuSign		
PPD PPD PPD 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: 03-Sep-2020 | 08:28 Viewed: 03-Sep-2020 | 14:57 Signed: 03-Sep-2020 | 14:58
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Signer Events	Signature	Timestamp
PPD PPD PPD 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: 03-Sep-2020 | 08:28 Viewed: 03-Sep-2020 | 08:47 Signed: 03-Sep-2020 | 08:47
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	03-Sep-2020 | 08:28
Certified Delivered	Security Checked	03-Sep-2020 | 14:57
Signing Complete	Security Checked	03-Sep-2020 | 14:58
Completed	Security Checked	03-Sep-2020 | 14:58

Payment Events	Status	Timestamps