Statistical Analysis Plan LOXO-BTK-20008

A Phase I, Open Label, Fixed-sequence Drug Interaction Study to Investigate the Effect of Multiple Oral Doses of LOXO-305 on the Pharmacokinetics of a Single Dose of Intravenous and Oral Midazolam (CYP3A4 Substrate) in Healthy Subjects

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LIST OF ABBREVIATIONS

Abbreviations pertain to the statistical analysis plan (SAP) only (not the tables, figures, and listings [TFLs]).

ADaM	analysis data model
AE	adverse event
AUC_{0-24}	area under the concentration-time curve from hour 0 to 24 hours postdose
AUC_{0-inf}	area under the concentration-time curve from time 0 extrapolated to infinity
AUC_{0-t}	area under the concentration-time curve from hour 0 to the last measurable concentration
AUC_{tau}	area under the concentration-time curve during a dosing interval
$\%AUC_{extrap}$	percentage extrapolation for area under the concentration-time curve extrapolated to infinity
BLQ	below the limit of quantification
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CL	apparent total clearance
CL/F	apparent systemic clearance
$\bar{C}L_{ss}/\bar{F}$	apparent systemic plasma clearance at steady state
C_{max}	maximum observed plasma concentration
C_{trough}	concentration observed at the end of a dosing interval
CP 1	endogenous coproporphyrin I
CP 3	endogenous coproporphyrin III
C_{ss}	steady state concentration
CRU	Clinical Research Unit
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
CYP	cytochrome P450
ECG	electrocardiogram
EOS	end of study
EOT	end of treatment
ET	early termination
Geom CV	geometric CV
GLSM	geometric least squares mean
Geom Mean	geometric mean
GMR	geometric mean ratios
HDYF	How Do You Feel?

ICF	Informed Consent Form
ICH	International Council for/Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IV	intravenous
λ_z	apparent terminal elimination rate constant
ln	natural log
LSM	least squares mean
MedDRA	Medical Dictionary for Regulatory Activities
MRAUC	Metabolite-to-parent AUC ratio
NC	not calculated
NR	no result
OATP1B1	organic anion transporting polypeptide 1B1
OATP1B3	organic anion transporting polypeptide 1B3
PK	pharmacokinetic(s)
QD	once daily
R_{AUC}	accumulation ratio
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
$t_{1/2}$	apparent terminal elimination half-life
TEAE	treatment-emergent adverse event
TFL	table, figure, and listing
t_{max}	time to maximum observed concentration
UA	urinalysis
V_z	volume of distribution
V_z/F	apparent volume of distribution
V_{ss}	volume of distribution at steady state
WHODrug	World Health Organization Drug Dictionary

1. INTRODUCTION

This SAP has been developed after review of the clinical study protocol (Final Version 2.0 dated 11 September 2020) and electronic case report form.

This SAP describes the planned analysis of the pharmacokinetic (PK), and safety and tolerability data from this study. A detailed description of the planned TFLs to be presented in the clinical study report (CSR) is provided in the accompanying TFL shells document.

In general, the analyses are based on information from the protocol, unless they have been modified by agreement with Loxo Oncology, Inc. A limited amount of information about this study (eg, objectives, study design) is given to help the reader's interpretation. This SAP must be finalized prior to the lock of the clinical database for this study. When the SAP and TFL shells are approved, they will serve as the template for this study's CSR.

This SAP supersedes any statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified accordingly in the CSR. Any substantial deviations from this SAP will be agreed with Loxo Oncology, Inc. and identified in the CSR.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonisation (ICH) E9 guideline *Statistical Principles for Clinical Trials* and ICH E3 guideline *Structure and Content of Clinical Study Reports*.[1,2]

The document history is presented in Appendix 1.

2. STUDY OBJECTIVES

2.1. Primary Objectives

The primary objectives of this study are:

- to assess the effect of multiple oral doses of LOXO-305 on the PK of a single oral dose of midazolam in healthy subjects
- to assess the effect of multiple oral doses of LOXO-305 on the PK of a single intravenous (IV) dose of midazolam in healthy subjects

2.2. Secondary Objectives

The secondary objectives of this study are:

- to assess the safety and tolerability of multiple oral doses of LOXO-305 when administered alone, coadministered with a single oral dose of midazolam and coadministered with a single IV dose of midazolam.
- to assess the PK of single and multiple doses of LOXO-305 when given alone or with midazolam in healthy subjects.

2.3. Exploratory Objective

The exploratory objective of this study is:

- to assess the effect of a single oral dose of LOXO-305 and steady state LOXO-305 on the PK of endogenous coproporphyrins I and III (CP 1 and 3) as biomarkers of the organic anion transporting polypeptide 1B1 (OATP1B1) and 1B3 (OATP1B3) inhibition in healthy subjects.

3. STUDY ENDPOINTS

3.1. Primary Endpoints

The following PK parameters will be calculated whenever possible, based on the plasma concentrations of midazolam and its metabolite 1-OH-midazolam following oral and IV single dose administration (as appropriate):

- area under the concentration-time curve from hour 0 to the last measurable concentration (AUC_{0-t})
- area under the concentration-time from time 0 extrapolated to infinity (AUC_{0-inf})
- percent extrapolation for AUC_{0-inf} ($\%AUC_{extrap}$)
- maximum observed concentration (C_{max})
- time to maximum concentration (t_{max})
- apparent terminal elimination rate constant (λ_z)
- apparent systemic clearance (CL/F; oral midazolam)
- total clearance (CL; IV midazolam)
- apparent plasma terminal elimination half-life ($t_{1/2}$)
- apparent volume of distribution (V_z/F; oral midazolam)
- volume of distribution (V_z) (IV midazolam)
- volume of distribution at steady state (V_{ss}; IV midazolam).

3.2. Secondary Endpoints

The following PK parameters will be calculated whenever possible, based on the plasma concentrations of LOXO-305 following single and multiple dose administration (as appropriate):

- AUC_{0-t}
- AUC during a dosing interval (AUC_{tau})
- C_{max}
- concentration observed at the end of the dosing interval (C_{trough})

- t_{max}
- CL/F at steady state ($CL_{,ss}/F$)
- accumulation ratio (R_{AUC}).

Safety and tolerability will be assessed by monitoring adverse events (AEs), performing physical examinations and clinical laboratory evaluations, measuring vital signs and oxygen saturation by continuous pulse oximetry, and performing 12-lead electrocardiograms (ECGs) and concomitant medications.

3.3. Exploratory endpoints

The following PK parameters will be calculated whenever possible, based on the plasma concentrations of coproporphyrins I and III following single dose and steady-state LOXO-305 (as appropriate): C_{max}, t_{max}, AUC from time 0 to 24 hours (AUC_{0-24}), and steady state concentration (C_{ss}).

4. STUDY DESIGN

This is a Phase 1, open-label, 2-period, fixed-sequence drug-drug interaction study to investigate the effect of multiple oral doses of LOXO-305 on the PK of a single oral dose of midazolam and on the PK of a single IV dose of midazolam in healthy subjects. The study will also assess the single and multiple oral dose PK of LOXO-305 and the effect of a single oral dose of LOXO-305 and the effect of LOXO-305 at steady state (following multiple dose administration) on the PK of endogenous coproporphyrins I and III as biomarkers of OATP1B1 and OATP1B3 inhibition in healthy subjects.

In Period 1, Day 1, a single 250 µg (0.25 mL of 1-mg/mL solution) IV bolus dose of midazolam will be administered over approximately 30 seconds in the morning following a 10-hour fast prior to dosing and a 4-hour fast postdose. On Day 3, a single 500 µg (0.25 mL of 2-mg/mL syrup) oral dose of midazolam will be administered in the morning following a fast of at least 10 hours prior to dosing and 4 hours postdose. On Day 3, the oral midazolam dose will be administered alone at the actual time of the Day 1 IV midazolam dose (± 1 hour). Blood samples for concentration of midazolam, 1-hydroxymidazolam (1-OH-midazolam), and coproporphyrins I and III in plasma will be collected on CCI [redacted]

In Period 2 (starting on Day 5), oral doses of 200 mg LOXO-305 will be administered once daily for 13 consecutive days (Days 5 through 17) in the morning. On Days 5, 14, 15, and 17 oral doses of 200 mg LOXO-305 will be administered following a fast of at least 10 hours prior to dosing and at least 4 hours postdose. On Day 6 through 13, and Day 16 oral doses of 200 mg LOXO-305 will be administered following a fast of at least 2 hours prior to dosing and 1-hour postdose, with the exception of days where clinical laboratory evaluations are performed. On Days 5 through 14, and Day 16, 200 mg LOXO-305 will be administered alone at the actual time of the Day 1 IV midazolam dose (± 1 hour). On the morning of Day 15, an oral dose of 200 mg LOXO-305 will be administered 2 hours (± 15 minutes) prior to administering a single 250 µg (0.25 mL of 1-mg/mL solution) IV bolus dose of midazolam. On the morning of Day 17, an oral dose of 200 mg LOXO-305 will be

administered first before a single oral 500 µg (0.25 mL of 2-mg/mL syrup) dose of midazolam is administered (within 5 minutes). On Days 15 and 17, LOXO-305 will be administered at the actual time of the Day 1 IV midazolam dose (± 1 hour). Blood samples for concentration of LOXO-305 and coproporphyrins I and III in plasma will be collected on CCI . Blood samples for concentration of midazolam, 1-OH-midazolam, and LOXO-305 in plasma CCI . Additionally, blood samples for concentration of LOXO-305 in plasma will be collected on CCI

There will be a washout period of 2 days between the IV dose of midazolam and the oral dose of midazolam on Days 1 and 3 (Period 1) and Days 15 and 17 (Period 2), respectively.

To assess their eligibility to enter the study, potential subjects will be screened within 28 days (Days -29 to -2) and be admitted to the Clinical Research Unit (CRU) on Day -1 (Check-in). Subjects will be confined at the CRU from the time of Check-in (Day -1) until End of Treatment (EOT) on Day 21 upon completion of all PK and safety assessments or Early Termination (ET) if the subject discontinues. A follow-up phone call will occur for all subjects who received at least 1 dose of study drug (including subjects who are terminated early) 7 days (± 2 days) after EOT or ET. The duration of participation is expected to be approximately 59 days (Screening through follow-up phone call).

Replacement subjects may be enrolled only if deemed necessary by the Sponsor.

The start of the study is defined as the date the first subject who is enrolled in the study signs an Informed Consent Form (ICF). Note that enrolled subjects are defined as those subjects who are assigned a dose of study drug; this definition excludes screen failure subjects. Study completion is defined as the time of the last subject's follow-up call.

In this study, physical examinations, 12-lead ECGs, vital sign measurements, oxygen saturation measured by continuous pulse oximetry, How Do You Feel? (HDYF?) inquiries, clinical chemistry panel, coagulation parameters, hematology panel, urinalysis (UA) and recording of concomitant medications will be performed at specified times during the study.

The AEs and serious adverse events (SAEs) will be collected beginning at informed consent. The AEs will be reported throughout the study (ie, from signing of the ICF until End of Study [EOS], or until ET if the subject discontinues from the study and does not complete a follow-up phone call), either as subject medical history (if the event is reported as beginning prior to signing of the ICF or if the event occurs prior to study drug administration on Day 1 and is assessed as not related to study procedures by the Investigator [or designee]) or as AEs (if the event occurs after signing of the ICF but prior to study drug administration on Day 1 and is assessed as related to study procedures by the Investigator [or designee], or if the event occurs after study drug administration on Day 1 through EOT or ET regardless of relationship to study drug). From EOT or ET through EOS, only AEs assessed as related to study drug by the Investigator (or designee) are to be reported. All SAEs that develop from the time of ICF signing until EOS (or ET, if the subject discontinues from the study and does not complete a follow-up phone call) are to be reported.

5. SAMPLE SIZE JUSTIFICATION

Up to CCI [redacted] adult male and female subjects (women of non-childbearing potential only) will be enrolled. This is a Phase 1 study and the sample size is not based upon any formal power calculations but is consistent with previous studies of a similar design. Up to CCI [redacted] are anticipated to be sufficient to provide a reliable estimate of the magnitude and variability of the interaction.

Every attempt will be made to enroll at least 4 subjects of each sex in the study.

6. STUDY TREATMENTS

The study treatment sequence name and ordering to be used in the TFLs are presented in Table 1.

Table 1: Presentation of Study Treatment Sequence in TFLs

Study Treatment Sequence	Order in TFLs
250 µg IV midazolam (Day 1) / 500 µg oral midazolam (Day 3) / 200 mg LOXO-305 (Day 5-Day 14) / 200 mg LOXO-305 + 250 µg IV midazolam (Day 15) / 200 mg LOXO-305 (Day 16) / 200 mg LOXO-305 + 500 µg oral midazolam (Day 17)	1

The study treatment names, and ordering to be used in the TFLs are presented in Table 2, Table 3 and Table 4.

Table 2: Presentation of Study Treatments in Safety summary TFLs

Period	Study Treatment	Order in TFLs
1	250 µg IV midazolam	1
1	500 µg oral midazolam	2
2	200 mg LOXO-305	3
2	200 mg LOXO-305 + 250 µg IV midazolam	4
2	200 mg LOXO-305 + 500 µg oral midazolam	5

Table 3: Presentation of Study Treatments in PK summary TFLs

Period	Study Treatment	Order in TFLs
1	250 µg IV midazolam	1
1	500 µg oral midazolam	2
2	200 mg LOXO-305 QD Day (Day 5)	3
2	200 mg LOXO-305 QD (Days 6 through 14, Day 16)	4
2	200 mg LOXO-305 + 250 µg IV midazolam	5
2	200 mg LOXO-305 + 500 µg oral midazolam	6

Table 4: Presentation of Study Treatments in PK CP 1 and 3 summary TFLs

Period	Study Treatment	Order in TFLs
1	Prior to Administration of LOXO-305 (Days 1 and 15)*	1
2	200 mg LOXO-305 QD (Day 5)	2
2	200 mg LOXO-305 QD (Days 6 through 14, Day 16)	3

**On Day 1 and Day 15 where CP 1 and 3 PK is assessed, subjects were also dosed with 250 ug IV midazolam; however, the study treatment has been labelled in relation to the dosing of LOXO-305 as the comparison of interest is CP 1 and 3 in the absence of LOXO-305 versus CP 1 and 3 in the presence of single and multiple doses of LOXO-305*

All treatments described above are the planned treatments. The TFLs will reflect the actual treatments received.

7. DEFINITIONS OF POPULATIONS

Any protocol deviations, including those related to COVID-19, will be considered prior to database lock for their importance and taken into consideration when assigning subjects to populations.

7.1. All Subjects Population

The all subjects population will include all subjects who signed the ICF and had any study assessment recorded in the database per the protocol.

7.2. Safety Population

The safety population will include all subjects who received at least 1 dose of study drugs (LOXO-305, IV midazolam, or oral midazolam). Subjects will be classified into groups based on actual treatment received.

7.3. Pharmacokinetic Population

The PK population will include all subjects who received at least 1 dose of any study drug (LOXO-305, IV midazolam, or oral midazolam), have at least 1 quantifiable PK concentration, and for whom at least 1 PK parameter can be computed. A subject may be excluded from the PK summary statistics and statistical analysis if the subject has an AE of vomiting that occurs at or before 2 times the median t_{max}.

8. STATISTICAL METHODOLOGY

8.1. General

Listings will be provided for all data captured in the database. Listings will include all subjects assigned to the all subjects population and include data up to the point of study completion or discontinuation. Subjects are generally considered to have completed the study if they completed all protocol-specified procedures and assessments for the EOT visit. Any

subject who discontinued the study will be identified accordingly in the listings. Summaries and statistical analyses will include the subjects assigned to the relevant population based on data type.

Data analysis will be performed using the SAS® statistical software package Version 9.4 (or higher if upversioned during the study).

Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) ADaM Version 2.1 (or higher if upversioned during the study) and CDISC ADaM Implementation Guide Version 1.2 (or higher if upversioned during the study). Pinnacle 21 Community Validator Version 2.2.0 (or higher if upversioned during the study) will be utilized to ensure compliance with CDISC standards.

Caution should be used when interpreting results from the statistical analyses conducted in this study because the sample size is not based on power calculations.

Where reference is made to 'all calculations', this includes, but is not limited to, summary statistics, statistical analyses, baseline derivation, and changes from baseline.

8.1.1. Calculation of the Summary Statistics

For continuous data the following rules will be applied:

- Missing values will not be imputed, unless specifically stated otherwise.
- Unrounded data will be used in the calculation of summary statistics.
- If the number of subjects with valid observations (n) <3, summary statistics will not be calculated, with the exception of n, minimum, and maximum.
- As ET data is not associated with any scheduled timepoint, it will be excluded from all calculations of summary statistics.
- Post dose repeats and unscheduled assessments will not be included in calculation of summary statistics.

For categorical data the following rules will be applied:

- If the categories of a parameter are ordered (eg, AE severity), all categories between the possible minimum and maximum categories will be included, even if n = 0 for a given category. If the categories are not ordered (eg, race), only those categories for which there is at least 1 subject represented will be included.
- Missing values will not be imputed, with the exception of AEs where the 'worst-case' approach will be taken (see Section 8.6.1), or unless specifically stated otherwise. A 'missing' category will be included for any parameter for which information is missing. This will ensure that the population size totals are consistent across different parameters.

All protocol deviations and data issues (eg missing data, out of protocol window) that occur during the study, including those related to COVID-19, will be considered prior to database lock for their severity/impact on how the data will be displayed.

8.1.2. Repeat and Unscheduled Assessments

For vital sign measurements and 12-lead ECG data only, any predose value recorded in addition to the original value or a postdose value recorded within 15 minutes of the original value will be defined as a repeat value; any postdose value recorded more than 15 minutes after the original value will be defined as an unscheduled value. For all other data types (eg, clinical laboratory values), any value recorded in addition to the original value will be defined as an unscheduled value.

The original scheduled value will be used in all calculations post dose. In the event of any repeats or unscheduled measurements taken pre-dose the values will be considered when determining the baseline value for each period.

Post dose repeats, unscheduled assessments, and ET measurements will be excluded from all calculations, with the exception of the baseline derivation (see Section 8.1.3).

8.1.3. Definitions of Baseline and Change from Baseline

Baseline value is defined as the last non missing measurement before administration of study drug in each period.

Period 1

In general, baseline for period 1 refers to the pre-dose value collected on Day 1 period 1. For clinical laboratory evaluations, baseline is the value collected on Day -1 period 1.

Period 2

In general, baseline for period 2 refers to the pre-dose value collected on Day 5 period 2. For clinical laboratory evaluations, baseline is the value collected pre-dose on Day 3 period 1.

If the date/time of the value is incomplete or missing, it will be excluded from the baseline calculation, unless the incomplete date/time indicates the value was recorded prior to dosing with study drug in Period 1 or Period 2.

Individual changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the timepoint. The mean change from baseline will be defined as the mean of the individual changes from baseline for all subjects.

See Section 8.1.2 for more detail on handling repeat and unscheduled readings in the calculations.

8.2. Subject Disposition and Population Assignment

Subject disposition and population assignment will be listed.

A summary table by study treatment sequence will be provided, based on the all subjects population.

8.3. Demographics and Baseline Characteristics

The demographics and baseline characteristics including age, sex, race, ethnicity, height, body weight, and body mass index will be listed.

A summary table by study treatment sequence will be provided, based on the safety population.

8.4. Prior and Concomitant Medication

Prior medication will be defined as medication that ends prior to the first dose of Midazolam IV. Concomitant medication will be defined as medication that starts during or after the first dose of Midazolam IV or starts but does not end prior to the first dose of Midazolam IV.

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODrug) Global, Format B3, Version March 2020. Prior and concomitant medications will be listed.

8.5. Pharmacokinetic Assessments

8.5.1. Pharmacokinetic Analysis

The following PK parameters will be determined where possible from the plasma concentration data for the following analytes using noncompartmental methods in validated software program Phoenix WinNonlin (Certara, Version 8.1 or higher):

Midazolam and metabolite 1-OH-midazolam (following IV and oral doses, unless otherwise specified)

Parameter	Units[a]	Definition
AUC_{0-t}	h*ng/mL	area under the concentration-time curve from hour 0 to the time of last measurable concentration (t_{last})[b]
AUC_{0-inf}	h*ng/mL	area under the concentration-time curve from hour 0 extrapolated to infinity[c]
$\%AUC_{extrap}$	%	percentage extrapolation for AUC_{0-inf}
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time to maximum observed concentration
λ_z	1/h	apparent terminal elimination rate constant
$t_{1/2}$	h	terminal elimination half-life
CL/F	L/h	apparent systemic clearance (midazolam only, following oral dose)
CL	L/h	total clearance (midazolam only, following IV dose)
V_z/F	L	apparent volume of distribution during the terminal phase (midazolam only, following oral dose)
V_z	L	volume of distribution (midazolam only, following IV dose)

V_{ss}	L	volume of distribution at steady state (midazolam only, following IV dose)
MRAUC		AUC_{0-inf} ratio of 1-OH-midazolam to midazolam [d]

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] Area under the concentration-time curve will be calculated using the linear trapezoidal rule for increasing and decreasing concentrations.
[c] Based on the last observed measurable concentration
[d] If AUC_{0-inf} cannot be determined reliably for all subjects or analytes, an alternative AUC measure, such as AUC to a fixed time point or AUC_{0-t}, may be used

LOXO-305 (following single and multiple dose administration, unless otherwise specified)

Parameter	Units[a]	Definition
AUC_{0-t}	h*ng/mL	area under the concentration-time curve from hour 0 to t_{last}[b]
AUC_{tau}	h*ng/mL	area under the concentration-time curve during a dosing interval[b]
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time to maximum observed concentration
CL_{ss}/F	L/h	apparent systemic clearance at steady state (after multiple doses; days 14, 15, and 17)
C_{trough}	ng/mL	Concentration observed at the end of a dosing interval (predose concentrations on Days 6 through 17 and at 24 hours after Day 17 dose)
R_{AUC}	NA	Accumulation ratio (ratio of Day 14 AUC_{tau} to Day 5 AUC_{tau})

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] Area under the concentration-time curve will be calculated using the linear trapezoidal rule for increasing and decreasing concentrations.
NA = not applicable

Coproporphyrins I and III (following single dose midazolam (IV administration), single dose LOXO-305, and steady-state LOXO-305, as appropriate, unless otherwise specified)

Parameter	Units[a]	Definition
AUC_{0-24}	h*ng/mL	area under the concentration-time curve from hour 0 to 24 hours[b]
C_{max}	ng/mL	maximum observed concentration

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] Area under the concentration-time curve will be calculated using the linear trapezoidal rule for increasing and decreasing concentrations.

Additional PK parameters may be determined where appropriate.

PK analysis will be carried out where possible using actual blood sampling times postdose. If an actual time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

The parameters C_{max}, and t_{max} will be obtained directly from the concentration-time profiles. If C_{max} occurs at more than 1 time point, t_{max} will be assigned to the first occurrence of C_{max}.

8.5.1.1. Criteria for the Calculation of Apparent Terminal Elimination Rate Constant and Half-life

The start of the terminal elimination phase for each subject will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in concentrations.

The apparent terminal elimination rate constant (λ_z) will only be calculated when a reliable estimate can be obtained using at least 3 data points, preferably not including C_{max}, and the adjusted coefficient for determination of exponential fit (R^2-adj) of the regression line is ≥0.7. Parameters requiring λ_z for their calculation (eg, $AUC_{0\text{-inf}}$, $t_{1/2}$, CL, CL/F, V_{ss}, V_z, and V_z/F) will only be calculated if the R^2-adj value of the regression line is ≥0.7.

The following regression-related diagnostic PK parameters will be determined, when possible:

Parameter	Units	Definition
λ_z Upper	h	end of exponential fit
λ_z Lower	h	start of exponential fit
λ_z N	NA	number of data points included in the log-linear regression
λ_z Span Ratio	NA	time period over which λ_z was determined as a ratio of $t_{1/2}$
R^2-adj	NA	adjusted coefficient for determination of exponential fit

Where possible, the span of time used in the determination of λ_z (ie, the difference between λ_z Upper and λ_z Lower) should be ≥2 half-lives. If the λ_z Span Ratio is <2, the robustness of the $t_{1/2}$ values will be discussed in the CSR.

8.5.1.2. Criteria for Calculation and Reporting of Area Under the Concentration-time Curve

The minimum requirement for the calculation of area under the concentration-time curve (AUC) will be the inclusion of at least 3 consecutive concentrations above the lower limit of quantification. If there are only 3 consecutive concentrations, at least 1 should follow C_{max}. An exception may be made for metabolites, where C_{max} may be the last time point.

If the extrapolated area is >30%, $AUC_{0\text{-inf}}$ (and derived parameters) may be excluded from the summary statistics and statistical analysis at the discretion of the sponsor or pharmacokineticist.

If $AUC_{0\text{-inf}}$ cannot be determined reliably for all subjects or treatments, an alternative AUC measure, such as AUC to a fixed time point or $AUC_{0\text{-t}}$, may be used in the statistical analysis of drug interaction.

8.5.1.3. Criteria for Handling Below the Limit of Quantification or Missing Concentrations for Pharmacokinetic Analysis

Plasma concentrations below the limit of quantification (BLQ) will be assigned a value of 0 before the first measurable concentration and thereafter BLQs will be treated as missing. The following rules apply with special situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.
- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.
- If a predose plasma concentration is missing prior to an initial drug treatment, it may be set to zero by default within Phoenix WinNonlin, other than for CP 1 and CP 3, which will be set to missing.

8.5.1.4. Treatment of Outliers in Pharmacokinetic Analysis

If a value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude the value from the PK analysis. However, the exclusion of any data must have strong justification and will be documented in the CSR.

Any quantifiable predose concentration value for the first dose of a specific study drug within the treatment period will be considered anomalous and set to missing for the PK analysis.

If the predose concentration of midazolam or 1-OH-midazolam is >5% of C_{max} in the second, all PK concentration and parameter data will be excluded from the summary statistics and statistical analysis for that period.

8.5.2. Presentation of Pharmacokinetic Data

If the actual time of sample collection deviates from the nominal time by more than ±10%, the concentration will be flagged and excluded from the summary statistics. Individual concentrations deemed to be anomalous will be flagged in the listings and excluded from the summary statistics.

For PK concentration data the following rules will apply:

- Values that are BLQ will be set to 0 for the calculation of summary statistics.
- Arithmetic mean or median values that are BLQ will be presented as 0.
- If any BLQ results (treated as 0) are in a series of summarized data, geometric mean and coefficient of variation (CV) of geometric mean will be reported as not calculated (NC).

For PK parameters the following rule will apply:

- Geometric mean and coefficient of variation will not be calculated for t_{max}.

PK analysis will use actual times as recorded on the electronic case report form.

8.5.2.1. Presentation Pharmacokinetic Parameters

For the calculation of summary statistics of PK parameters, all not reported (NR) and Not Calculated (NC) values in a data series will be set to missing.

The AUC values will be set to NC if they have been calculated using fewer than 3 concentrations, and/or 3 concentrations if the last is C_{max}.

8.5.3. Pharmacokinetic Statistical Methodology

All PK concentrations and parameters will be listed.

Summary tables, mean (+ standard deviation [SD]) figures, overlaying individual figures, and individual figures by treatment and time postdose will be provided for plasma PK concentrations. All PK concentration figures will be produced on both linear and semi-logarithmic scales, with the exception of figures across all days, which will be produced on the linear scale only. The +SD bars will only be displayed on the linear scale.

Summary tables by treatment will be provided for all PK parameters, with the exception of diagnostic regression-related PK parameters. Summary statistics (n, Mean, SD, CV, minimum, median, maximum, geometric mean [Geom Mean] and geometric CV [Geom CV]) will be calculated for plasma LOXO-305, Midazolam, 1-OH-Midazolam and CP 1 and CP 3PK parameters. Excluded subjects will be listed in the PK parameter tables, but will be excluded from the summary statistics and noted as such in the tables.

8.5.3.1. Statistical Analyses

8.5.3.1.1. Primary PK endpoint

A statistical analysis will be conducted to investigate the drug-drug interaction on the PK of midazolam and 1-OH midazolam for AUC_{0-t}, AUC_{0-inf}, and C_{max}. The comparisons of interest are:

To assess the impact of multiple oral doses of LOXO-305 on the single IV dose PK of midazolam (AUC_{0-t}, AUC_{0-inf}, and C_{max})

- 200 mg LOXO-305 co-administered with 250 µg IV midazolam (Day 15) (test treatment) vs 250 µg IV midazolam (Day 1) (reference treatment).

To assess the impact of multiple oral doses of LOXO-305 on the single oral dose PK of midazolam and 1-OH midazolam (AUC_{0-t}, AUC_{0-inf}, and C_{max})

- 200 mg LOXO-305 co-administered with 500 µg Oral midazolam (Day 17) (test treatment) vs 500 µg Oral midazolam (Day 3) (reference treatment).

The natural log (ln)-transformed[3] midazolam and 1-OH-midazolam PK parameters (AUC_{0-t}, AUC_{0-inf}, and C_{max}) will be analyzed using a mixed model.[4] The model will include actual treatment as fixed effect, and subject as a random effect.

For each PK parameter separately, the least squares mean (LSM) for each treatment, difference in LSMs between the test and reference treatments, and corresponding 90% confidence interval (CI) will be calculated; these values will then be exponentiated to give the geometric least squares mean (GLSM), geometric mean ratios (GMR), and corresponding 90% CI.

Additionally, the pooled estimate (across all treatments) of the within-subject CV will be calculated, and residual plots will be produced to assess the adequacy of the model(s) fitted.

Examples of the SAS code that will be used are as follows:

Mixed Model Analysis

```
proc mixed data = <data in>;

  class treatment usubjid;
  model lpk = treatment / cl residual ddfm = kr;
  lsmeans treatment / cl pdiff = control('1') alpha = 0.1;
  random intercept / subject = usubjid;
  ods output lsmeans = <data out>;
  ods output diffs = <data out>;
  ods output covparms = <data out>;
run;
```

8.5.3.1.2. Secondary PK endpoints

A steady state analysis will be performed on the ln-transformed C_{trough} concentrations for LOXO-305 using Helmert contrasts[5]. C_{trough} concentrations for LOXO-305 will be calculated at the end of a dosing interval (predose concentrations on Days 6 through 17 and at 24 hours after Day 17 dose), Days 6 through to Day 14 will be investigated for steady state. The model will include profile day and subject will be fitted as a repeated measure.

Helmert contrasts are constructed such that each timepoint is compared to the mean of the subsequent timepoints, i.e the pre-dose day 6 C_{trough} concentration is compared to the mean C_{trough} concentrations of Days 7 through to Day 14, a non-significant p-value indicates that steady state has been achieved.

Steady State Analysis

```
proc mixed data = <data in>;
  class usubjid profileday;
  model lpk = profileday;
  repeated profileday / subject = usubjid;
  estimate 'Pre dose Day 6 vs mean of predose days 7-14'
           1 -1/8 -1/8 -1/8 -1/8 -1/8 -1/8 -1/8 -1/8;
  estimate 'Pre dose Day 7 vs mean of predose days 8-14'
           0  1 -1/7 -1/7 -1/7 -1/7 -1/7 -1/7 -1/7;
  estimate 'Pre dose Day 8 vs mean of predose days 9-14'
           0  0  -1  -1/6 -1/6 -1/6 -1/6 -1/6 -1/6;
  ........
run;
```

8.5.3.1.3. Exploratory PK endpoint

A statistical analysis will be conducted to investigate the effect of LOXO-305 on the PK of endogenous coproporphyrins I and III AUC_{0-24} and C_{max}. The comparisons of interest are:

To assess the impact of single oral doses of LOXO-305 on the PK of endogenous coproporphyrins I and III (AUC_{0-24} and C_{max}).

- 200 mg LOXO-305 (Day 5) (test treatment) vs Prior to Administration of LOXO-305 (Day 1) (reference treatment).

To assess the impact of multiple oral doses of LOXO-305 (steady state) on the PK of endogenous coproporphyrins I and III (AUC_{0-24} and C_{max}).

- 200 mg LOXO-305 Steady State (Day 15) (test treatment) vs Prior to Administration of LOXO-305 (Day 1) (reference treatment).

The same mixed model approach outlined in section 8.5.3.1.1 will be used.

8.6. Safety and Tolerability Assessments

8.6.1. Adverse Events

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.0. All AEs will be assigned severity grade using National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

A treatment-emergent adverse event (TEAE) will be defined as an AE that starts during or after the first dose of midazolam IV, or starts prior to the first dose and increases in severity after the first dose.

A treatment-related TEAE will be defined as a TEAE with a relationship of related to the study treatment (LOXO-305 or midazolam [Oral or IV]), as determined by the investigator.

- A TEAE occurring during or after Day 1 dosing and prior to Day 3 dosing will be assigned to 250 ug IV MDZ
- A TEAE occurring during or after Day 3 dosing and prior to Day 5 dosing will be assigned to 500 ug oral MDZ
- A TEAE occurring during or after Day 5 dosing and prior to Day 15 dosing will be assigned to 200 mg LOXO-305
- A TEAE occurring during or after Day 15 dosing and prior to Day 17 dosing will be assigned to 200 mg LOXO-305 + 250 µg IV midazolam
- A TEAE occurring during or after Day 17 dosing will be assigned to 200 mg LOXO-305 + 500 µg oral midazolam

All AEs will be listed. In addition to the data recorded in the database, the listings will include derived onset time and duration. Onset time will be calculated from the time of the last dose for TEAEs only.

The frequency of subjects with TEAEs and the number of TEAEs will be summarized for the following categories:

- TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- TEAEs by severity and treatment
- Treatment-related TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- Treatment-related TEAEs by severity and treatment

The frequency of subjects will be summarized separately for TEAEs and treatment-related TEAEs by the following:

- System organ class, preferred term, and treatment
- Preferred term and treatment
- System organ class, preferred term, day of onset, and treatment

For the AE data the following rules will apply:

- For the derivation of TEAE status: If the start date/time of an AE is incomplete or missing, an AE will be assumed to be a TEAE, unless the incomplete start date/time or the end date/time indicates an AE started prior to the first dose.
- For the derivation of treatment-related TEAE status: If the study treatment relationship for a TEAE is missing, a TEAE will be assumed to be a treatment-related TEAE.
- For the derivation of onset time: If the start date/time of an AE is missing, onset time will not be calculated. If the start date/time of an AE is incomplete, where possible, the minimum possible onset time will be calculated and presented in '≥DD:HH:MM' format (eg, if the date/time of the last dose is 01MAY2019/08:00 and recorded start date/time of an AE is 03MAY2019, then the minimum possible onset time will be calculated by assuming the AE started at the first hour and minute of 03MAY2019 [03MAY2019/00:00], thus will be presented as onset time ≥01:16:00 in the listing).
- For the derivation of duration: If the end date/time of an AE is missing, duration will not be calculated. If the start or end date/time of an AE is incomplete, where possible, the maximum possible duration will be calculated and presented in '≤DD:HH:MM' format (eg, if the start of an AE date/time is 01MAY2019/08:00 and its recorded end date/time is 03MAY2019, then the maximum possible duration will be calculated by

assuming the AE ended at the last hour and minute of 03MAY2019 [03MAY2019/23:59], thus will be presented as duration ≤02:15:59 in the listing).

- For the calculation of summary statistics: If the severity of a TEAE is missing, a TEAE will be counted under the maximum severity possible, up to Grade 4 in the absence of a fatal outcome.

- For the calculation of summary statistics: If a subject experienced multiple TEAEs with the same preferred term for the same treatment, this will be counted as 1 TEAE for that treatment under the maximum severity recorded.

8.6.2. Clinical Laboratory Evaluations

All clinical laboratory evaluations, with changes from baseline, will be listed; any value outside the clinical reference range will be flagged, and whether the out of range value was deemed clinically significant or not clinically significant will be indicated. Separate listings will be provided for any parameter for which there is any individual subject value outside the respective clinical reference range.

The observed results and change from baseline for clinical chemistry, hematology, and coagulation parameters will be summarized descriptively by study part and timepoint.

Values recorded as <x, ≤x, >x, or ≥x will be displayed in the listings as recorded. For the derivation of listing flags, calculation of summary statistics, and presentation in the figures, <x and ≤x values will be set to 0 whereas >x and ≥x values will be set to x.

8.6.3. Vital Signs Results

All vital sign results with changes from baseline will be listed; any value outside the clinical reference range will be flagged, and whether the out of range value was deemed clinically significant or not clinically significant will be indicated.

The observed results and change from baseline for all vital signs parameters will be summarized descriptively by treatment and timepoint.

8.6.4. Pulse Oximetry

Pulse oximetry with changes from the pre-midazolam dose value will be listed.

The observed results and change from the pre-midazolam dose value of pulse oximetry will be summarized descriptively on Days 1, 3, 15, and 17.

8.6.5. 12-lead Electrocardiogram Parameters

All 12-lead ECG parameters with changes from baseline will be listed; any value outside the clinical reference range will be flagged, and whether the out of range value was deemed clinically significant or not clinically significant will be indicated.

The observed results and change from baseline for all 12-lead ECG parameters will be summarized descriptively by treatment group and timepoint. QTcF values that are > 450 msec and increase from baseline > 30 msec will be flagged in the data listing.

8.6.6. Other Assessments

Medical history and physical examination will be listed. Any physical examination abnormalities reported will also be flagged as clinically significant or not clinically significant as indicated.

All other safety and tolerability assessments not detailed in the above sections will be listed only.

8.6.7. Safety and Tolerability Statistical Methodology

No inferential statistical analyses are planned.

9. INTERIM ANALYSES

No interim analyses are planned.

10. CHANGES FROM THE PROTOCOL-SPECIFIED ANALYSES

There were no changes from the protocol-specified analyses.

11. REFERENCES

1. ICH. ICH Harmonised Tripartite Guideline: Statistical principles for clinical trials (E9). 5 February 1998.

2. ICH. ICH Harmonised Tripartite Guideline: Structure and content of clinical study reports (E3). 30 November 1995.

3. Keene ON. The log transformation is special. *Stat Med*. 1995;14(8):811-819.

4. Brown H, Prescott R. *Applied Mixed Models in Medicine*. Chichester: John Wiley & Sons, 1999.

5. 16. Chow SC, Liu JP. Design and analysis of bioavailability and bioequivalence studies. New York: Marcel Dekker; 1992.

12. APPENDICES

Appendix 1: Document History

Status, Version	Date of Change	Summary/Reason for Changes
Final, Version 1.0	NA	NA; the first version.

NA = not applicable