EicOsis Human Health, Inc.

EC5026-1-01

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A Single-Center, Double-Blinded, Placebo-Controlled, Phase 1A Single Ascending Dose Study to Investigate the Safety, Tolerability, and Pharmacokinetics of Sequential Dose Regimens of Oral EC5026 in Healthy Male and Female Subjects

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Statistical Analysis Plan

Final Version 1.0

Prepared by:

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List of Abbreviations

λz	apparent terminal elimination rate constant
$\%AUC_{extrap}$	percentage of the area extrapolated for calculation of $AUC_{0\text{-}inf}$
Ae	amount of drug excreted unchanged in the urine
AE	adverse event
ANOVA	analysis of variance
$AUC_{0\text{-}48}$	area under the serum concentration-time curve from time 0 to 48 hours postdose
$AUC_{0\text{-}t}$	area under the curve from time 0 to time t
$AUC_{0\text{-}\infty}$	extrapolation of the area under the curve from time 0 to infinity
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BLQ	below the limit of quantification
BMI	body mass index
BP	blood pressure
C_{last}	the last quantifiable plasma drug concentration
CI	confidence interval
CL	total serum clearance
CL/F	apparent total body clearance
CLr	renal clearance
C_{max}	maximum (peak) serum drug concentration
CSR	clinical study report
CRU	clinical research unit
ECG	electrocardiogram
eCRF	electronic case report form
EicOsis	EicOsis Human Health, Inc.
EOS	end of study
ET	early termination
FDA	Food and Drug Administration
Fe%	fraction of dose excreted in urine
$Fe\%_{t1\text{-}t2}$	fraction of the administered dose excreted in urine over each collection interval
GLP	Good Laboratory Practice
HR	heart rate
Kel	apparent terminal elimination rate constant
MedDRA	Medical Dictionary for Regulatory Activities
PD	pharmacodynamic
PK	pharmacokinetic(s)
PT	preferred term
QTcF	QT interval corrected for heart rate using Fridericia's formula

RR	time elapsed between 2 consecutive R waves as measured by electrocardiogram
Rsq	r^2, the coefficient of determination (goodness of fit statistic)
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SoA	schedule of activities
SOC	system organ class
SRC	Safety Review Committee
$t_{1/2}$	apparent terminal half life
TEAE	treatment-emergent adverse event
T_{max}	time to reach maximum observed serum concentration
ULN	upper limit of normal
V_z/F	apparent volume of distribution

1. Introduction

This document outlines the statistical methods to be implemented during the analysis of data collected within the scope of EicOsis, protocol EC5026-1-01 (A Single-Center, Double-Blinded, Placebo-Controlled, Phase 1A Single Ascending Dose Study to Investigate the Safety, Tolerability, and Pharmacokinetics of Sequential Dose Regimens of Oral EC5026 in Healthy Male and Female Subjects), Version 2.0, 15 October 2019.

This is a Phase 1A, first-in-human study with EC5026 and is designed to provide initial safety, tolerability, and pharmacokinetics (PK) data regarding EC5026 for future clinical studies. Analysis of urine and plasma concentrations of EC5026 will characterize the single-dose PK of EC5026 and help to refine the dosing strategy for subsequent multiple-dose studies.

The purpose of this statistical analysis plan is to define the planned statistical analysis of the study data consistent with the study objectives.

2. Objectives

2.1. Primary Objectives

The primary objectives of this study are as follows:

- To investigate the safety and tolerability of escalating dose regimens of EC5026 administered once orally in healthy male and female subjects.
- To investigate the PK of escalating dose regimens of EC5026 administered once orally in healthy male and female subjects.

2.2. Exploratory Objectives

The exploratory objectives of this study are as follows:

- To investigate the response of various biomarkers to a single dose of EC5026 administered to healthy male and female subjects.
- To investigate putative metabolites of EC5026 following single oral administration of EC5026 in healthy male and female subjects.

3. Investigational Plan

3.1. Overall Study Design and Plan

This is a Phase 1A, first-in-human, randomized, single-center, double-blind, placebo-controlled, single ascending dose (SAD) study to investigate the safety, tolerability, and PK of EC5026 in healthy male and female subjects.

This study will have up to 5 cohorts with a total of up to 40 subjects (8 subjects per cohort). An optional sixth dose cohort (maximum 48 subjects) will be considered after completion of 5 dose cohorts.

The study will consist of a screening period (Day -28 to -2), check-in (Day -1), study drug administration (Day 1), treatment period (Days 1 to 7), clinic visit (Day 7), and a follow-up/end-of-study (EOS) visit (Day 14) for each cohort.

In each cohort, 6 subjects will be randomly assigned to receive EC5026 and 2 subjects will receive placebo. Subjects will be enrolled in a manner to include approximately equal number of male and female subjects. The doses will be escalated in a stepwise fashion following acceptable safety and tolerability of the preceding dose(s).

A blinded sentinel group of 2 subjects will be dosed at least 2 days before the remaining 6 subjects will receive blinded doses of active study drug or placebo. If either of the sentinel subjects experiences a serious adverse event (SAE) or an adverse event (AE) that fulfills the stopping rule criteria as discussed in protocol Section 9.2.1.1, the dosing will be stopped for all subjects in the study pending a safety review; the remaining subjects will not initiate dosing until the safety incident is resolved.

The planned dose levels are provided in Table 3-1.

Table 3-1 Planned Dosing Levels

Cohort	Dose	Number of Subjects Receiving Study Drug	Number of Subjects Receiving Placebo
1A (Sentinel Group)	0.5 mg	1	1
1B	0.5 mg	5	1
2A (Sentinel Group)	2 mg	1	1
2B	2 mg	5	1
3A (Sentinel Group)	8 mg	1	1
3B	8 mg	5	1
4A (Sentinel Group)	16 mg	1	1
4B	16 mg	5	1
5A (Sentinel Group)	32 mg	1	1
5B	32 mg	5	1
6A (Sentinel Group)[a]	48 mg	1	1
6B[a]	48 mg	5	1

[a] Optional sixth dose cohort

Subjects will be confined to the clinical unit from check-in (Day -1) until discharge on

Day 5. A clinic visit will occur on Day 7, and a follow-up visit (EOS visit) will occur on Day 14. The duration of the study, including a 28-day screening period, is approximately 43 days.

Safety and PK data through Day 7, after completion of each cohort, will be reviewed by the safety review committee (SRC) in a blinded fashion before escalation to the next dose cohort.

Safety, PK, and biomarker endpoints will be evaluated in the study.

3.2. Study Endpoints

3.2.1. Pharmacokinetic Endpoints

The following PK parameters for EC5026 will be calculated as endpoints using standard noncompartmental methods: $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}48}$, C_{max}, T_{max}, K_{el}, $t_{1/2}$, CL/F, and V_z/F. The following urine PK parameters for EC5026 will be calculated as endpoints using standard noncompartmental methods: CL_R, Ae, $Ae_{(t1\text{-}t2)}$, and Fe%.

3.2.2. Safety Endpoints

The safety and tolerability endpoints will include monitoring and recording of AEs, clinical laboratory test results (hematology, coagulation, serum chemistry, and urinalysis), vital sign measurements, 12-lead electrocardiogram (ECG) results, ECG telemetry, physical examination findings, and treatment emergent adverse events (TEAEs) compared with EC5026 blood levels.

3.2.3. Pharmacodynamic Endpoints

Exploratory biomarker assessments will be determined by EicOsis and reported separately from the clinical study report (CSR).

4. General Statistical Considerations

All data collected will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings but not included in the calculation of summary statistics.

Unless otherwise indicated, outputs which are summarized by treatment will be summarized for each dose level for subjects on active study drug; subjects who receive placebo will be pooled into a single group.

For categorical variables, frequencies and percentages will be presented. Continuous variables will be summarized using descriptive statistics (number of subjects, mean, median, standard deviation [SD], minimum, and maximum).

Unless specified otherwise, baseline will be defined as the last nonmissing assessment prior to the study drug administration. Unscheduled visits will be used in determining baseline.

Unless otherwise indicated, outputs which are summarized by treatment will be summarized for each dose level for subjects on active study drug; subjects who receive placebo will be pooled into a single group.

Study day will be calculated relative to the first dose date as follows:

- If assessment date is on or after the dose of study drug administration, then Study Day = Assessment Date – Dose Date + 1
- Otherwise, Study Day = Assessment Date – Dose Date

4.1. Sample Size

This study will enroll up to 48 subjects in 6 cohorts of 8 subjects each (the sixth cohort is optional). The sample size (N = 40 or N=48 with inclusion of the sixth cohort) for this study is based on clinical and practical considerations and not on a formal statistical power calculation. The sample size is considered sufficient to evaluate the objectives of the study.

4.2. Analysis Populations

The safety population will include all subjects who receive a single dose of study drug.

The PK population will include subjects who receive a single dose of EC5026 and have sufficient concentration data to support accurate estimation of at least 1 PK parameter. Subjects who experience vomiting within 2 times the median T_{max} after study drug dosing will be excluded from the PK analysis.

The pharmacodynamic (PD) population will include subjects who receive a single dose of EC5026 and have a postdose PD measurement. Exploratory biomarker assessments will be determined by EicOsis and reported separately from the CSR and will not be described in this analysis plan.

5. Subject Disposition, Protocol Deviations, and Follow-up

5.1. Disposition

Subject disposition will be summarized by treatment and total for all randomized subjects.

The number of subjects who are randomly assigned in the study and the number and percentage of subjects who complete the study will be presented. Frequency and percentage of subjects who withdraw or discontinue from the study, and the reason for withdrawal or discontinuation, will also be summarized.

Subject disposition data, analysis populations, and randomization data will be presented in data listings. Subjects with screen failure will also be presented in a data listing.

5.2. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the protocol. An important deviation (sometimes referred to as a major or significant deviation) is a subset of protocol deviations that leads to a subject being discontinued from the study, or significantly affects the subject's rights, safety, or well-being or the completeness, accuracy, and reliability of the study data. An important deviation can include nonadherence to inclusion or exclusion criteria or nonadherence to Food and Drug Administration regulations or ICH E6(R2) guidelines.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. The Investigator will be notified in writing by the monitor of deviations. The Institutional Review Board should be notified of all protocol deviations, if appropriate, in a timely manner.

Important protocol deviations will be summarized for all randomized subjects.

All protocol deviations will be presented in a data listing, including the categorization of the deviation as important or not. Details of admission criteria deviations will be presented in a separate data listing.

6. Demographics and Baseline Characteristics

6.1. Demographics

Demographic and baseline characteristics will be summarized by treatment and total for the safety population.

The demographic characteristics consist of age (years), sex, race, and ethnicity. The baseline characteristics consist of baseline weight (kg), height (cm), and body mass index (kg/m^2). Percentages will be based on the total number of subjects in the safety population.

Subject demographic and baseline characteristics will be presented in a data listing.

6.1.1. Medical History

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, version to be delineated in CSR).

Medical history will be presented in a data listing for the safety population.

7. Treatments, Medications, and Meals

7.1. Prior and Concomitant Medications

Information regarding prior medications taken by the subject within the 30 days before signing the informed consent form will be recorded in the subject's electronic case report form (eCRF).

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the investigator. If a concomitant medication is taken, except for those specified in the protocol, a joint decision will be made by the investigator and the sponsor to continue or discontinue the subject based on the time the medication was administered, its pharmacology and PK, and whether the use of the medication will compromise the safety of the subject or the interpretation of the data. The investigator is responsible for ensuring that details regarding the medication are adequately recorded in the eCRF.

Prior and concomitant medications and therapies will be coded using the latest version of the World Health Organization Drug Dictionary.

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

7.2. Medical and Surgical Treatment Procedures

All medical and surgical treatment procedures will be coded using MedDRA (version to be delineated in the CSR). All medical and surgical treatment procedures will be presented in a data listing for the safety population.

7.3. Study Drug Administration

All doses of study drug will be administered in the clinical unit under direct observation of site personnel and recorded in the eCRF. Clinic personnel will confirm that the subject has received the entire dose of study drug. The date and time of study drug dosing will be recorded on the appropriate page of the eCRF. If a subject is not administered study drug, the reason for the missed dose will be recorded.

All study drug administration data will be presented in a data listing.

8. Pharmacokinetic Analysis

8.1. Data Handling

Plasma concentrations that are below the limit of quantification (BLQ) will be treated as zero for calculation of descriptive statistics. When all concentrations are BLQ for a timepoint, mean BLQ concentrations will be presented as BLQ, and the SD and CV will be reported as not applicable, otherwise the calculated mean will be presented.

For PK parameter calculations, BLQ values will be treated as zero with the exception that a BLQ value between 2 quantifiable concentrations will be set as missing. If consecutive BLQ concentrations are followed by quantifiable concentrations in the terminal phase, those concentrations after BLQ concentrations will be treated as missing.

Missing concentrations will be excluded from summaries and PK parameter calculations.

8.2. Plasma Concentrations

Blood samples for the determination of plasma concentrations of EC5026 will be collected on Day 1 at the following time points: predose (0), and at 1.25, 2.25, 4.25, 6.25, 8.25, 12.25, 24, 36, 48, 72, 84, 96, and 108 hours postdose. Additional blood samples will be obtained on the mornings of Days 7 (168 hours) and 14 (336 hours) (or ET) postdose.

Pharmacokinetic collections that have an actual sampling time that deviates from the nominal collection time by more than 10% will be flagged in the data listing, but the nominal sampling time will be used for summarization.

EC5026 plasma concentration data will be summarized using descriptive statistics (number of subjects, number of nonmissing values, arithmetic mean, SD, CV, median, minimum, and maximum) by dose group and nominal sampling time. Individual and mean EC5026 plasma concentration versus time data will be plotted by dose group. For ease of presentation, mean plasma concentrations of EC5026 will be plotted by nominal time by dose group on both linear and semilogarithmic scales.

Plasma PK concentrations of EC5026 will be reported to the precision of the raw data in the data listing presentations; summary statistics for arithmetic mean, median, minimum, maximum, and SD will be reported to 3 significant figures; and CV% will be reported to 1 decimal place.

8.3. Urine Concentration

Urine will be collected for analysis of EC5026 during the following time intervals: before dosing and 0 to 8 hours, 8 to 16 hours, 16 to 24 hours, 24 to 32 hours, 32 to 40 hours, and 40 to 48 hours after administration of EC5026.

Individual urine concentrations of EC5026 will be presented in a data listing by time interval and dose group.

8.4. Plasma Pharmacokinetic Parameters

The plasma concentration-time data for EC5026 will be analyzed by non-compartmental analysis using Phoenix® WinNonlin® (Certara USA, Inc., Princeton, NJ) Version 8.0 or higher. Actual sampling times will be used for the estimation of all plasma PK parameters.

If data allow, the following PK parameters will be calculated:

PK Parameter	Definition
C_{max}	Maximum (peak) plasma drug concentration
T_{max}	Time to reach maximum (peak) plasma concentration following drug administration
AUC_{0-t}	Area under the curve from time 0 to time t, where t is the time of the last quantifiable concentration calculated using the linear trapezoidal method.
AUC_{0-inf}	Extrapolation of the area under the curve from time 0 to infinity, calculated as $AUC_{0-inf} = AUC_{0-t} + C_{last}/\lambda_z$, where C_{last} is the last quantifiable plasma drug concentration, calculated using the linear trapezoidal method
AUC_{0-48}	Area under the curve from time 0 to time 48 hours postdose, calculated using the linear trapezoidal method.
$t_{1/2}$	Terminal half-life, calculated as: $t_{1/2} = \ln(2)/K_{el}$
CL/F	Apparent total clearance of the drug from plasma after oral administration, calculated as: $CL/F = Dose/AUC_{0-inf}$
Vz/F	Apparent volume of distribution

In addition to the previously defined PK parameters, which will be listed and summarized, the following parameters will also be listed to document the selection of data points used to estimate $t_{1/2}$ using non-compartmental procedures:

PK Parameter	Definition
$\%AUC_{extrap}$	Percentage of the area extrapolated for calculation of $AUC_{0-\infty}$.
K_{el}	Apparent terminal elimination rate constant, where K_{el} is the magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase.
Number of points	Number of data points used to estimate K_{el}; a minimum of 3 data points must be used, and C_{max} must not be included.
K_{el} lower	Lower bound used for the estimation of K_{el}.
K_{el} upper	Upper bound used for the estimation of K_{el}.
Rsq	r^2, the coefficient of determination (goodness of fit statistic); visual inspection of the terminal slope will be performed. In general, K_{el} may only be retained if $r^2 \geq 0.80$

Plasma PK parameters for EC5026 will be presented in data listings and summarized by dose group using descriptive statistics (number of subjects, arithmetic mean, SD, CV%, median, minimum, and maximum) by dose group. Geometric means and geometric CV% will be reported for AUC_{0-t}, AUC_{0-inf}, AUC_{0-48}, and C_{max}. T_{max} will be summarized using the descriptive statistics median, minimum, and maximum only.

Plasma PK parameters of EC5026 will be displayed to 3 significant figures in all data listings and summary tables, with exception of time variables (T_{max}, K_{el} lower, and K_{el} upper) which will be displayed to 2 decimal places.

8.5. Urine Pharmacokinetic Parameters

The urine concentration-time data for EC5026 will be analyzed by non-compartmental analysis using Phoenix® WinNonlin® (Certara USA, Inc., Princeton, NJ) Version 8.0 or higher or SAS Version 9.4 or higher (SAS Institute Inc., Cary, North Carolina).

If data allow, the following urine PK parameters will be calculated:

PK Parameter	Definition
Ae_{t1-t2}	Amount of drug excreted in urine (Ae) over each collection interval
Ae	Total amount of EC5026 excreted in urine
$Fe\%_{t1-t2}$	The fraction of the administered dose excreted in urine over each collection interval
Fe%	The fraction of the administered dose excreted in urine
CLr	Renal clearance

Urine PK parameters will be presented in data listings and summarized using descriptive statistics (n, arithmetic mean, SD, CV%, median, geometric mean, geometric CV%, minimum, and maximum) by dose group.

Urine PK parameters will be displayed to 3 significant figures in all data listings and summary tables.

8.6. Pharmacokinetic Statistical analysis

Dose-proportionality for EC5026 will be evaluated for $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}48}$, and C_{max}. A power model will be fitted to describe the relationship between Y ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}48}$, and C_{max}) and X (dose) using the least-squares linear regression model, $\ln(Y) = \ln(\alpha) + \beta \ln(X)$, which is the logarithmic form of $Y = \alpha X^{\beta}$.

The intercept of regression line, $\ln(\alpha)$, and the slope of the regression line, β, will be presented along with the 90% confidence interval (CI) of the slope. Dose-proportionality will be concluded if the 90% CI of the slope β lies entirely within $[1+\ln(0.8)/\ln(r), 1+\ln(1.25)/\ln(r)]$, where r is a ratio that describes the dose range and is defined as the ratio of highest dose/lowest dose (Smith et al 2000). If dose proportionality is not observed, then the lowest dose followed by the highest dose in succession will be removed, and the model will be fit again after the removal of a dose until dose proportionality is achieved or less than 3 dose groups remain.

The statistical analyses will be based on the PK population.

9. Pharmacodynamics

Exploratory PD assays may include biomarker assessments and plasma epoxide:diol ratios to evaluate potential target engagement of EC5026 versus the soluble epoxide hydrolase (sEH) enzyme. Exploratory PD assays will be reported separately and are not included in the statistical analysis plan.

10. Safety Analysis

All safety summaries and analyses will be conducted for the safety population.

10.1. Adverse Events

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment.

A TEAE is defined as any event not present before exposure to study drug or any event already present that worsens in intensity or frequency after exposure.
Only TEAEs will be included in summary tables.

Adverse events will be coded by preferred term (PT) and system organ class (SOC) using MedDRA (version to be delineated in the CSR).

A subject may have more than 1 AE for an SOC or PT. A subject with 2 or more AEs within the same level of summarization will be counted only once in that level. Percentages will be based upon the number of subjects in the safety population for each treatment group.

All AEs will be presented in a data listing.

10.1.1. Incidence of Adverse Events

An overview of AEs will be presented by treatment and total, including number and percentage of subjects with any:

- Treatment-emergent AE
- Treatment-related TEAE
- Moderate TEAE
- Treatment-related moderate TEAE
- Severe TEAE
- Treatment-related severe TEAE
- Serious TEAE
- Treatment-related serious TEAE
- Treatment-emergent AE leading to early discontinuation
- Death

All TEAEs will be presented in a summary table by treatment and total for each SOC and PT. Percentages will be calculated out of the number of subjects in the safety population.

10.1.2. Relationship of Adverse Events to Study Drug

The relationship or association of the study drug in causing or contributing to the AE will be characterized by the investigator as “Related” or “Not Related” as defined in protocol Section 9.3.5.

All TEAEs will be presented in a summary table for each treatment group and total by relationship to study drug. If a subject has 2 or more TEAEs in the same SOC (or with the same PT) with a different relationship to study drug, then a “Related” event will be used

for that subject. If the relationship information is missing, the AE will be considered related in the summary but will be presented as missing in the data listings.

10.1.3. Severity of Adverse Event

All AEs (laboratory and clinical symptoms) will be graded for severity. The severity will be classified as mild, moderate, or severe using the following criteria:

- Mild (Grade 1): Events that are transient and may require only minimal or no treatment or therapeutic intervention and do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.
- Potentially life-threatening Grade 4): An event that results in an emergency room visit or hospitalization.

All TEAEs will be summarized for each treatment group and total by maximum severity. If the severity information is missing, the AE will be considered severe in the summary but will be presented as missing in the data listings.

10.1.4. Serious Adverse Events

An AE or suspected adverse reaction is considered an SAE/suspected unexpected serious adverse reaction if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- Congenital anomaly or birth defect

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an

emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be presented in a data listing.

10.1.5. Adverse Events Leading to Study Drug Discontinuation

An AE where the answer to "What action was taken with study treatment?" is "Drug Withdrawn" will be considered an AE leading to study drug discontinuation.

All AEs leading to study drug discontinuation will be presented in a data listing.

10.2. Clinical Laboratory Evaluations

Clinical laboratory testing will occur at Screening, Check-in and on Days 1, 2, 3, 4, 5, 7 and 14/EOS. A complete list of assessments is provided in Appendix 2.

Actual results and changes from baseline values for hematology, coagulation, serum chemistry, and urinalysis test results will be summarized by visit and treatment for subjects in the safety population.

Shift from baseline in hematology, coagulation, serum chemistry, and urinalysis test results relative to the reference range will be summarized by visit and treatment using the frequency count and percentage of subjects in each category for the safety population.

Serology, urine drug screen, and pregnancy test data will not be summarized but will be available in the data listings.

All laboratory data will be presented in a data listing for the safety population.

10.3. Vital Sign Measurements and Weight

Vital signs will be measured at Screening, Check-in, on Day 1 within 45 minutes predose and at 0.5, 1, 2, 3, 4, 6, 8, and 12 hours following study drug dosing after the subject has been in the seated position for at least 5 minutes. On Days 2, 3, 4, 5, 7, and 14/EOS, evaluations will be performed in the morning. Vital signs will include blood pressure, pulse rate, respiratory rate, and body temperature.

Actual values and changes from baseline for vital sign data will be summarized by visit and treatment for subjects in the safety population. Changes from baseline to each scheduled post-baseline visit will be presented. Only systolic and diastolic blood pressure, pulse rate, respiratory rate and temperature will be included in the summary tables.

All vital sign, height, and weight measurements will be presented in a data listing.

10.4. Electrocardiograms

10.4.1. 12-Lead Electrocardiogram

Twelve-lead ECGs will be performed at Screening, Check-in, and on Day 1 within 45 minutes prior to study drug dosing (predose) and at 0.5, 1, 2, 3, 4, 6, 8, and 12 hours following study drug dosing after the subject has been in the supine position for at least 5 minutes. On Days 2, 3, 4, 5, 7, and 14/EOS, evaluations will be performed in the morning. A single repeat measurement is permitted at Screening for eligibility determination. Measurements of the following intervals will be reported: RR interval, PR interval, QRS width, QT interval, and QTcF. Assessments should include comments on whether the tracings are normal or abnormal; rhythm (if abnormal, specify the abnormality); presence of arrhythmia or conduction defects; morphology; any evidence of myocardial infarction; or ST-segment, T-Wave, and U-Wave abnormalities.

Actual values and changes from baseline for numeric ECG data will be summarized by visit and treatment for subjects in the safety population.

Shift from baseline in interpretation of ECG results will be summarized by visit and treatment using the frequency count and percentage of subjects in each category.

All 12-lead ECG data will be presented in a data listing for the safety population.

10.4.2. Electrocardiogram Telemetry

The ECG telemetry will be evaluated at Check-in and on Days 1, 2, 3, and 4. All ECG telemetry data will be presented in a data listing for the safety population.

10.5. Physical Examination

A full physical examination will be performed at Screening, Check-in (Day -1), and on Days 1, 2, 3, 4, 5, 7, and 14 (or ET). A full physical examination will include skin, head, ears, eyes, nose, throat, neck, thyroid, respiratory, neurological, cardiovascular, abdomen, lymph nodes, and musculoskeletal system/extremities.

Physical examination results will be presented in a data listing for the safety population.

11. Changes from the Planned Analysis

Subjects will not be stratified to male and female as proposed in protocol Section 3, but they will be enrolled to include approximately equal numbers of male and female.

12. Interim Analysis

No formal interim analyses will be performed in this study. A blinded safety data review will be performed for each cohort before dose escalation is allowed. An interim safety analysis will be performed on a blinded basis within 1 week following the end of each dosing sequence (ie, within 5 business days or 7 calendar days after each SAD sequence is administered). Placebo data will be pooled from all completed cohorts to create the placebo control for each successive interim dose comparison. Any SAE and a higher-than-placebo incidence of nonserious AEs will be evaluated on an ad hoc basis and presented to the sponsor for consideration before subjects will be enrolled in the next dose cohort.

The plasma concentration-time data and PK parameters for all subjects will be reviewed by the safety review committee (SRC). Pharmacokinetic stopping rules will be implemented based on the mean NOAEL of male and female animals from the most sensitive preclinical species considered for this study (28-day rat GLP safety study M386-18). Dose escalation will be stopped, and data will be reviewed by the SRC, if EC5026 C_{max} is equal to 920 ng/mL and/or if AUC_{0-48} is equal to 8400 ng•h/mL in any single subject within each cohort, or if the mean value is projected to reach either of these values in a subsequent cohort.

13. References

International Council for Harmonisation (ICH) E6 (R2) GCP: Integrated Addendum to ICH E6 (R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 [2018])
https://www.fda.gov/media/93884/download.

Protocol EC5026-1-01: A Single-Center, Double-Blind, Placebo-Controlled, Phase 1A Single Ascending Dose Study to Investigate the Safety, Tolerability, and Pharmacokinetics of Sequential Dose Regimens of Oral EC5026 in Healthy Male and Female Subjects, Version 2.0, 15 October 2019.

14. Appendices

Appendix 1 Schedule of Events

Phase	Screening	Check-in	Treatment Period						FU/EOS/ET
Procedure[Error! Reference source not found.] **Day**	**–28 to –2**	**–1**	**1**	**2**	**3**	**4**	**5**	**7**	**14 (±2)**
Admission to clinic		X							
Randomization			X						
Discharge from clinic							X		
Outpatient visit[(b)]								X	X
Informed consent	X								
Inclusion/exclusion criteria	X	X							
Demographics	X								
Medical and medication history	X	X							
Physical examination[(c)]	X	X	X	X	X	X	X	X	X
Height, weight, and BMI[(d)]	X	X	X						
Vital sign measurements[(e)]	X	X	X	X	X	X	X	X	X
12-lead ECG[(f)]	X	X	X	X	X	X	X	X	X
ECG telemetry[(f)]		X	X	X	X	X			
Clinical laboratory testing[(g)]	X	X	X	X	X	X	X	X	X
Viral serology[(h)]	X								
Urine drug and alcohol screen[(i)]	X	X							
Serum pregnancy test[(j)]	X	X							X
Serum FSH[(k)]	X								
EC5026 administration[(l)]			X						
PK blood sample collection[(m)]			X	X	X	X	X	X	X
Blood samples for external biomarker assessments[(n)]			X	X	X	X	X	X	X
Urine volume, electrolytes[(o)]		X	X	X	X	X			
Urine samples for EC5026[(p)]			X	X					
Fasting period[(q)]		X	X						
Non-fasting period[(r)]				X	X	X	X	X	
AEs[(s)]		X	X	X	X	X	X	X	X
Prior/concomitant medications	X	X	X	X	X	X	X	X	X

Abbreviations: AE, adverse event; BMI, body mass index; ECG, electrocardiogram; EOS, end of study; ET, early termination; FSH, follicle-stimulating hormone; FU, follow-up; PK, pharmacokinetic; QTcF, QT interval corrected for heart rate using Fridericia's formula.

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) Follow-up/EOS visit will occur on Day 14 (± 2 days) or 2 days after ET.

(c) A full physical examination will be performed at Screening, Check-in (Day -1), and on Days 1, 2, 3, 4, 5, 7, and 14 (or ET). A full physical examination will include skin, head, ears, eyes, nose, throat, neck, thyroid, respiratory, neurological, cardiovascular, abdomen, lymph nodes, and musculoskeletal system/extremities.
(d) Height and weight will be measured, and BMI calculated at Screening only. Only weight will be measured on Days –1 and 1.
(e) Vital signs will be measured at Screening, on Day –1, and on Day 1 within 45 minutes prior to study drug dosing (predose) and at 0.5, 1, 2, 3, 4, 6, 8, and 12 hours following study drug dosing after the subject has been in the seated position for at least 5 minutes. On other days, evaluations will be performed in the morning. Vital signs will include blood pressure, pulse rate, respiratory rate, and body temperature.
(f) Twelve-lead ECGs will be performed at Screening, on Day –1, and on Day 1 within 45 minutes prior to study drug dosing (predose) and at 0.5, 1, 2, 3, 4, 6, 8, and 12 hours following study drug dosing after the subject has been in the supine position for at least 5 minutes. On other days, evaluations will be performed in the morning. A single repeat measurement is permitted at Screening for eligibility determination. Measurements of the following intervals will be reported: RR interval, PR interval, QRS width, QT interval, and QTcF. Assessments should include comments on whether the tracings are normal or abnormal; rhythm (if abnormal, specify the abnormality); presence of arrhythmia or conduction defects; morphology; any evidence of myocardial infarction; or ST-segment, T-Wave, and U-Wave abnormalities.
(g) Clinical laboratory testing will occur at Screening, on Day –1, and on Days 1, 2, 3, 4, 5, 7, and 14 (or ET). A complete list of assessments is provided in Section 6.3.2. Blood and urine samples will be collected under fasted conditions and prepared per the clinic's standard procedures.
(h) Viral serology testing (Screening only) will include hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus types 1 and 2 antibodies.
(i) Urine drug and alcohol screen will occur at Screening and on Day –1 per the clinic's standard procedures.
(j) A serum pregnancy test will be performed at Screening, on Day –1, and Day 14 (or ET) in premenopausal women of childbearing potential or women who are surgically sterile.
(k) A serum FSH test will be performed at Screening to confirm postmenopausal status in older women.
(l) The time of study drug dosing will be called "0" hour in each period and is denoted with grey shading. Study drug will be administered with 240 mL of room temperature water. Subjects will maintain an upright (ie, seated or standing) position for at least 4 hours after dosing.
(m) Blood samples for PK analysis of EC5026 in plasma will be collected prior to study drug dosing and at 1.25, 2.25, 4.25, 6.25, 8.25, 12.25, 24, 36, 48, 72, 84, 96, and 108 hours after study drug dosing and after vital signs have been recorded. Additional blood samples will be obtained on the mornings of Days 7 and 14 (or ET). Postdose PK blood samples should be collected within ±5 minutes of the scheduled collection time for the first 8.25 hours, then within ±15 minutes of the scheduled collection time up to 24 hours, and within ±30 minutes of the scheduled collection time up to clinic discharge on Day 5. Pharmacokinetic samples may be collected at any time during the day on Days 7 and 14. The actual time of blood sampling will be recorded in the source documents and electronic case report form.
(n) Blood samples (0.1 mL frozen plasma from each PK sample) for external exploratory biomarker assessments will be collected, stored, and shipped in bulk to the sponsor for poststudy biomarker measurements.
(o) Total voided urine will be combined and collected over the following intervals: –16 to –8, –8 to 0, 0 to 8, 8 to 16, 16 to 24, 24 to 32, 32 to 40, 40 to 48, 48 to 56, 56 to 64, and 64 to 72 hours. Urinalysis on pooled samples includes sodium, potassium, calcium, magnesium, chloride, phosphate, and bicarbonate.
(p) Urine samples for PK analysis of EC5026 will be collected before study drug administration (predose) and collected and pooled over the following intervals: 0 to 8, 8 to 16, 16 to 24, 24 to 32, 32 to 40, and 40 to 48 hours after study drug administration. Additional aliquots from each period will be collected for exploratory urinary metabolite profiling; samples will be collected, stored, and shipped in bulk to the sponsor for poststudy evaluation.

(q) During fasting periods, subjects should have nothing to eat or drink, except water, from 10 hours prior to EC5026 dosing until 4 hours after dosing. Water is permitted as desired except for the period 1 hour before and 1 hour after administration of study drug (other than as permitted for study drug dosing).
(r) During nonfasting periods, subjects should receive standardized meals per the clinic's standard procedures that are scheduled at approximately the same time each day.
(s) Adverse events will be assessed from the time of EC5026 dosing until Day 14 (or ET) and should be followed until they are resolved, stable, or judged by the investigator to be not clinically significant.

Appendix 2 CLINICAL LABORATORY ASSESSMENTS

The following clinical laboratory assessments will be performed:

Hematology	Complete blood count including red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cell count, differential white blood cell count, and platelet count
Coagulation	Activated partial thromboplastin time, prothrombin time, and international normalized ratio
Serum chemistry	Albumin, ALP, ALT, AST, bicarbonate, total bilirubin, blood urea nitrogen, calcium, chloride, total cholesterol, creatinine, gamma-glutamyltransferase, glucose, lactate dehydrogenase, phosphorus, potassium, total protein, sodium, magnesium, total protein, CPK, and uric acid
Urinalysis	Protein, glucose, blood, microalbumin, and microscopy under reflex only
Serology	Hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus antibody types 1 and 2 (Screening only)
Urine drug screen	Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine metabolites, cotinine, methamphetamines, methylenedioxymethamphetamine, and opioids including heroin, codeine, and oxycodone) and alcohol screen
Pregnancy test (female subjects only)	Serum follicle-stimulating hormone test for postmenopausal females and serum pregnancy test for premenopausal females

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatine phosphokinase.