Statistical Analysis Plan

A PHASE 1, OPEN-LABEL, SINGLE-DOSE, PARALLEL-GROUP STUDY TO EVALUATE THE EFFECTS OF RENAL IMPAIRMENT ON THE PHARMACOKINETICS OF ELX-02

Sponsor Study No. EL-008
inVentiv Health Clinique Inc. Project No. 182023

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Sponsor Study No.: EL-008

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Study Title: A Phase 1, Open-label, Single-Dose, Parallel-Group Study to Evaluate the Effects of Renal Impairment on the Pharmacokinetics of ELX-02

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LIST OF ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
$Ae_{interval}$	Amount of drug excreted in urine for each time interval, calculated as the urine concentration multiplied by the urine volume
Ae_{0-t}	Cumulative urinary excretion from time zero to time t, calculated as the sum of the amounts excreted over each collection interval.
AUC	Area under the curve
AUC_{0-24}	Area under the concentration-time curve from time zero to 24 hours post-dose
AUC_{0-72}	Area under the concentration-time curve from time zero to 72 hours post-dose
AUC_{0-t}	Area under the concentration-time curve from time zero to the last non-zero concentration
AUC_{0-inf}	Area under the concentration-time curve from time zero to infinity (extrapolated)
BLQ	Below lower limit of quantification
BMI	Body mass index
BSA	Body Surface Area
CI	Confidence interval
CL/F	Apparent Body Clearance
CL_R	Renal clearance, calculated as Ae_{0-24} / AUC_{0-24}
C_{max}	Maximum observed concentration
CPK	Creatine phosphokinase
CR/STBA Form	Confinement Report and Subjects To Be Analyzed Form
CSR	Clinical study report
CV	Coefficient of variation
D_{sc}	Dose administered by Subcutaneous
ECG	Electrocardiogram
eGFR	estimated glomerular filtration rate
ES	Estradiol

FDA	Food and Drug Administration
Fe_{0-t}	Fraction (% dose) excreted unchanged
GLM	Generalized linear model
HBsAg	Hepatitis B
HCV	Hepatitis C
HEENT	Head, eyes, ears, nose, and throat
HIV	Human Immunodeficiency Virus
HR	Heart rate
INR	International normalized ratio
IV	Intravenous
kg	Kilogram
K_{el}	Elimination rate constant
$K_{el\ Lower}$	The timepoint where K_{el} calculation begins.
$K_{el\ Upper}$	The actual sampling time of the last measurable concentration used to estimate the K_{el}.
L	Liters
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
MDRD	Modification of Diet in Renal Disease
mg	Milligram
Min	Minimum
mL	Milliliter
mmHg	Millimeters Mercury
MSE	Mean Square Error
PK	Pharmacokinetic
PR	PR interval
aPTT	Activated partial thromboplastin time
PT	Preferred term (for adverse events); Prothrombin time (for coagulation tests)
QT	QT interval
QTcF	Fridericia's corrected QT interval
RI	Renal Impairment

R_{max}	Maximum rate of urinary excretion, calculated by dividing the amount of drug excreted in each collection interval by the time over which it was collected
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SC	Subcutaneous
SD	Standard deviation
SOC	System Organ Class
SOPs	Standard operation procedures
TEAEs	Treatment-emergent adverse events
$T_{½\ el}$	Elimination half-life
T_{max}	Time of observed C_{max}
T_{Rmax}	Time of maximal urinary excretion, calculated as the midpoint of the collection interval during which R_{max} occurred
ULN	Upper limit of normal
V_d/F	Apparent Volume of Distribution
WHO DD	World Health Organization Drug Dictionary

1. Introduction

This statistical analysis plan (SAP) is intended to give a detailed description of the summaries and the analyses that will be generated for the present study by inVentiv or another statistical provider. Analyses specified in this plan are based on Eloxx Pharmaceuticals Study Protocol no. EL-008, Version Final 2.0, dated 06 Nov, 2018 (inVentiv Project No. 182023). Safety, tolerability, and pharmacokinetic (PK) analyses will all be described.

No change will be made without prior approval of the study Sponsor. No revision to the SAP is required for changes that do not affect the statistical analysis methods, definitions, or rules defined in this document. If changes made after the SAP has been finalized due to unforeseen circumstances and/or additional analyses are required to supplement the planned analyses described in the SAP, the changes and justification for the changes will be outlined in the clinical study report (CSR).

When applicable, all methodology and related processes will be conducted according to inVentiv's or another statistical provider's Standard Operating Procedures (SOPs) as appropriate.

Shells for all statistical tables, figures and listings referred to in this SAP will be displayed in a separate document.

2. Study Objective

2.1 Primary Objective

To determine the effect of various severities of renal impairment (RI) on the pharmacokinetics (PK) of ELX-02 following a single subcutaneous (SC) dose in subjects with normal renal function, mild, moderate, or severe renal impairment.

2.2 Secondary Objective

To assess the safety and tolerability of a single SC dose of ELX-02 in subjects with normal renal function, mild, moderate, or severe renal impairment.

3. Study Design

3.1 General Design

The study is a two-center, Phase 1, open-label, single dose, one-period, four-parallel-group, PK study in subjects with various severities of renal dysfunction and healthy volunteers. The study is intended for filing under the US Food and Drug Administration (FDA) regulations.

A total of approximately 24 to 26 healthy adult male or female volunteers will be enrolled.

Subjects will be enrolled into the trial and assigned to one of the 4 study groups. Six (6) subjects completing the study are targeted for each group of RI subjects (Groups 1 to 3) and 6 to 8 subjects will be included in the control group (Group 4). Attempts will be made to enroll at least two subjects of each gender in each group and subjects with various BMIs.

Subjects will be assigned to one of the following groups:

- Group 1 (Mild): 6 subjects with mildly decreased renal function: eGFR 60 - 89 mL/min/1.73 m^2;
- Group 2 (Moderate): 6 subjects with moderately decreased renal function: eGFR 30 - 59 mL/min/1.73 m^2;
- Group 3 (Severe): 6 subjects with severely decreased renal function: eGFR < 30 mL/min/1.73 m^2 and not requiring dialysis.
- Group 4 (Control): 6-8 healthy subjects with normal renal function: eGFR ≥ 90 mL/min/1.73 m^2;

Group 1 (Mild) and Group 2 (Moderate) will be dosed first, in parallel, with an interim analysis performed (refer to Section 7).

Subjects with normal renal function (Group 4) will be matched with renally impaired (RI) subjects by age (± 10 years), gender, and BMI (± 15%). A mean matching procedure will be performed. Therefore 6 subjects completing the study are targeted for each of the RI groups (Groups 1 to 3) and 6 to 8 subjects will be included in the control group (Group 4).

3.2 Study Procedures

The overall schedule of procedures and assessments is provided in the protocol.

3.3 Drug Administration

Each subject will be administered a single SC dose of 1 mg/kg ELX-02 (Eloxx Pharmaceuticals, USA) on Day 1. The body weight of each subject will be measured on Day -1 (check-in) and this value will be used to calculate the exact individual dose of ELX-02 required on a mg/kg basis. The study drug administration details (including treatment received, date and time of administration) will be listed by subject.

[redacted]

SC injection of ELX-02 will be done in the abdominal region around the umbilicus, by an appropriately qualified, GCP-trained, and experienced member of the study staff, as allowed by local, state, and institutional guidance. A study physician must be present during any ELX-02 administration.

3.4 Subject Withdrawal and Replacement

Subjects will be advised that they are free to withdraw from the study at any time. Over the course of the study, the Sponsor and the Investigator or a delegate may withdraw any subject from the study for one of the reasons described below; subject withdrawal will be done in accordance with clinical site SOP:

- Safety reason;
- Non-compliance with protocol requirements;
- Significant protocol deviation;
- Positive alcohol breath test, cotinine test (Group 4 only), drug screen, or pregnancy test;

Hematology, biochemistry, urinalysis, and coagulations results will be reviewed by a PI or a Sub-Investigator prior to dosing; subjects will be withdrawn from the study if it is deemed that the subject's safety may be at risk on the basis of these test results.

For subjects with RI (Groups 1 to 3) and taking medications that are allowed by the Principal Investigator, a positive result to opiate/benzodiazepine/THC screen (drug screen) may derive from the use of the concomitant medications. The subjects will not be automatically withdrawn from the study but will be evaluated on a case-by-case basis by the Principal Investigator. Subjects who withdraw or are withdrawn from the study after dosing will not be replaced. However, in the event that the number of drop-outs exceeds initial expectations, subjects who withdraw or are withdrawn might be replaced at the discretion of the Sponsor. Such replacement resulting in dosing more subjects than planned in this protocol would be documented in a protocol amendment.

Subjects who withdraw or are withdrawn will be asked to remain at the clinic until the PI or a delegate agrees that the subject is fine and can be discharged. As soon as subject withdrawal is confirmed, blood sampling will be stopped. A PK blood draw may be collected at the time of withdrawal if deemed required by the PI. Study exit procedures will be performed at the time of withdrawal from the study or as soon as possible thereafter.

4. Changes from the Protocol

Three changes in planned analyses were done compared to the protocol in this SAP: the PK primary endpoint of AUC_{0-72} was changed to AUC_{0-24} in order to better compare partial AUC across dose groups, the calculation of renal clearance (CL_R) was changed from Ae_{0-72}/AUC_{0-72} to $Ae_{0-24}/AUC_{0-24,}$ and the PK endpoint of T_{max} was changed from a secondary endpoint to a primary endpoint. AUC_{0-72} remains in the analysis as a secondary endpoint. In addition, a clarification was added to indicate that the pharmacokinetic statistical comparisons (i.e.: Mild vs. Control, Moderate vs. Control, and Severe vs. Control) would be done separately.

5. Primary and Secondary Endpoints

AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, C_{max} and T_{max} calculated using plasma ELX-02 concentration data and Ae_{0-t} and R_{max} calculated using urine ELX-02 concentration data will be the primary PK endpoints. All other PK parameters calculated for ELX-02 will be regarded as secondary endpoints (refer to Section 10.3).

Safety and tolerability parameters are secondary endpoints (refer to Section 9).

6. Analysis Populations

The analysis of safety and tolerability parameters will be based on the Safety population detailed in Section 6.1. The analysis of PK parameters will be based on the Pharmacokinetic population detailed in Section 6.2.

6.1 Safety Population

The safety population is defined as all subjects who received study medication.

6.2 Pharmacokinetic Population

The PK population will include all subjects completing the study and for whom the PK profile can be adequately characterized.

Any subject with ELX-02 pre-dose concentration prior to ELX-02 administration on Day 1 will be presented in the listings of concentrations and pharmacokinetic data but excluded from the descriptive statistics and analysis of variance (ANOVA) tables if the pre-dose concentration is greater than 5% of the C_{max} value measured on Day 1 for that subject. Subjects withdrawn due to AEs or PK reasons will be analyzed and reported but excluded from the statistical analysis. However, the concentrations for these subjects will be included in the descriptive statistics.

Here are some aspects to be considered (but not to be limited to) when determining data availability for the PK population: inclusion and exclusion criteria, acceptable times for visit dates and measurements, compliance with treatment, the nature and quality of the data, withdrawal, and any protocol deviation. The final responsibility of deciding which subjects are to be included or excluded lies with the principal investigator and/or the Sponsor.

Following to the end of the clinical portion of the study, prior to the start of the analysis of the samples, subjects analysed for PK population will be established by the inVentiv Health project team and finalized after Sponsor's review, according to the SOP ANI 3550_4 (Use of the Confinement Report and Subjects To Be Analyzed Form). All information including: subject withdrawals, adverse events that could affect characterization of the PK profile, concomitant medications taken, deviations to dosing procedures, deviations to study restrictions and sample particularities/missing blood draw will be detailed in the Confinement Report and Subjects To Be Analyzed Form (CR/STBA form).

7. Interim Analyses

No formal interim analysis was planned in the protocol. However, interim PK analyses will be conducted after completion of dosing of the mild (Group 1) and moderate (Group 2) RI patients in order to assess plasma and urine ELX-02 exposure prior to proceeding with administration to severe (Group 3) RI patients. In interim analyses, PK parameters will be calculated using scheduled sampling times as actual sampling times will not be available.

Mean ± SD plasma ELX-02 concentration versus time curves will be presented using linear and semi-log scales. Descriptive statistics for all PK ELX-02 primary and secondary parameters sorted by renal function group will be provided. Additional plots and/or analyses may be performed at the discretion of the kineticist.

8. Study Population and Exposure

Descriptive statistics will be used to present and summarize study population and exposure data.

8.1 Subject Disposition

Subject disposition will be summarized by group (frequency and the percentage of subjects) and overall. The following categories will be summarized by number and percentage.

- Screened and screen failures subjects (overall only).
- Enrolled and not enrolled subjects (overall only).
- Subject who are dosed (by group and overall).
- Subject who are not dosed (by group and overall).
- Subject who have completed the study (by group and overall).
- Subjects who discontinued in the treatment period (by group and overall).
- Primary reason for discontinuation (by group and overall).

Subject discontinuation information will be listed.

For subjects enrolled, not enrolled and screen failures, the percentage denominator will be the number of screened subjects. For all other calculations, the percentage denominator will be the number of subjects dosed in each group. For overall, percentages based on the overall number of subjects dosed (safety population).

8.2 Protocol Deviations

The protocol deviations will be categorized and listed by subject.

8.3 Demographics and Baseline Characteristics

Descriptive statistics (sample size (n), mean, median, standard deviation [SD], minimum [Min], and maximum [Max]) will be calculated for continuous variables (age, body mass index [BMI], height, and weight) considering last results (scheduled or unscheduled) obtained prior to the dosing of ELX-02. Frequency counts and percentages will be tabulated for categorical variables (age group, gender, ethnicity and race). All summaries will be presented by group and overall. All demographic characteristics will be listed by subject.

8.4 Renal Function Classification

Subjects will be classified according to their estimated glomerular filtration rate (eGFR) obtained from the Modification of Diet in Renal Disease (MDRD4) study equation (Table 8-1) based on serum creatinine. Cystatin concentrations may be used for data analysis if needed. Subjects' eligibility will be based on screening results and subjects will be assigned at check-in to one of the following groups based on the screening eGFR:

Table 8-1 Classification of Subjects in Groups According to Renal Function

Group	Description	eGFR (mL/min/1.73m^2)
1	Mild decrease in GFR	60-89
2	Moderate decrease in GFR	30-59
3	Severe decrease in GFR	< 30
4	Control (normal) GFR	≥ 90

MDRD4 (4-variable) equation:

$$eGFR = 175 \times (Scr)^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American}).$$

where: eGFR is expressed as mL/min/1.73 m^2 of body surface area.

Scr is serum creatinine expressed in mg/dL.

Age is expressed in years

Variables are: serum creatinine, age, gender, and race.

In addition, serum cystatin C will be measured during screening procedures. However, no additional analyses will be performed based on this endogenous glomerular filtration marker.

eGFR and its individual component results will be listed by subject.

8.5 Medical History

Medical history will be listed by subject. The Medical Dictionary for Regulatory Activities (MedDRA®) Version 21.1 will be used to classify all medical history findings by System Organ Class (SOC) and Preferred Term (PT).

8.6 Prior and Concomitant Medications

The use of prior and/or concomitant medications will be monitored throughout the study and listed by subject. The World Health Organization Drug Dictionary (WHO DD) Version Sep2018, format B3 will be used to classify all medication reported during the study.

A summary of prior medications use will be presented. Concomitant medications will be summarized by group and overall. All prior and concomitant medications will be listed by subject.

8.7 Study Drug Administration

The study drug administration details (including treatment received, date and time of administration) will be listed by subject.

Time of dosing will be set equal to the time when the ELX-02 injection is administered to the subject.

9. Safety Analyses

Safety and tolerability of ELX-02 will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study drug, outcome, duration, and management), local reactions at the injection site, clinical laboratory parameters (biochemistry, hematology, cogulation, and urinalysis), 12-lead electrocardiogram (ECG), vital sign, and physical examination. Laboratory values (hematology, biochemistry, urinalysis, and coagulation), will be summarized overall or according to the group, as appropriate.

Safety data will be summarized, but will not be subjected to inferential analysis.

9.1 Physical Examination Findings

A complete physical examination will be performed at screening. A brief physical examination will be done on Day -1, at check-out on Day 4 and at study exit.

The complete physical examination will include at least the following components: head, eyes, ears, nose, throat (HEENT), neck, chest, lungs, abdomen, musculoskeletal, dermatological, cardiovascular/peripheral vascular evaluation, and general neurological examination.

A brief physical examination includes assessments of the following components: HEENT, chest, lungs, abdomen, musculoskeletal, dermatological, cardiovascular/peripheral vascular, general neurological examination, and areas of note elicited from the subject.

Body measurements will be performed at screening and will include body weight, height measurement, and BMI. Body measurement will be summarized (mean, median, standard deviation [SD], minimum [Min], maximum [Max], and sample size) in demographic tables (safety and PK).

Any abnormal findings judged to be clinically significant will be documented as medical history or as an AE, depending upon time of observation, as appropriate. Any physical examination findings documented as AEs will be included in the AE summaries.

9.2 Adverse Events

Treatment-emergent AEs (TEAEs) and non-TEAEs will be listed. TEAEs will be defined as AEs that occur on or after the date and time of study drug administration. Any AE that first occurs pre-dose but worsens in severity after the first study drug administration will also be considered a TEAE. Non-TEAEs are those that occur prior to the first administration of the study medication and resolved prior to dosing or that first occur prior to the first study drug administration but do not worsen in severity after dosing. TEAEs will be captured during the study until study exit. Adverse events will be followed-up until complete resolution, or until the Principal Investigator or Medical Sub-Investigator judges safe to discontinue follow-up.

The incidence of TEAEs will be summarized using the safety population. The MedDRA® dictionary Version 21.1 will be used to classify all TEAEs reported during the study by SOC and PT.

The relationship of TEAEs will be classified according to the study protocol as certain, probably/likely possible, unlikely, or unrelated (not related) to ELX-02. The severity of TEAEs will be classified according to the study protocol as mild, moderate or severe.

Incidence of subjects who experienced TEAEs (frequency and the percentage of subjects) will be presented for each group and overall by:

- SOC and PT;
- SOC, PT, and relationship;
- SOC, PT, and maximum severity.

Each subject may only contribute once to each of the incidence rates, for a TEAE occurring during the same group, regardless of the number of occurrences; the highest severity or highest relationship will be presented, as appropriate. In each table, the SOC will be presented in descending order of overall incidence rate in terms of frequency of subjects and then in frequency of events (alphabetical order will be used in case of equal rates). For each SOC, PT will be presented the same way.

Number of TEAEs will also be presented by:

- SOC and PT;
- SOC, PT, and relationship;
- SOC, PT, and maximum severity.

Serious adverse events (SAEs) and TEAEs leading to early discontinuation of study drug will be listed separately.

9.3 Laboratory Parameters

Biochemistry will be performed at screening, at check-in on Day -1, and at study exit, following a fasting period of at least 8 hours. The following will be assessed: albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, calcium, chloride, glucose, phosphorus, potassium, creatinine, sodium, direct bilirubin, indirect bilirubin, total bilirubin, creatine phosphokinase (CPK), and total protein. Considering that indirect bilirubin is calculated from total and direct bilirubin values, indirect bilirubin result would not be available in case of direct bilirubin below the limit of quantification. Serum creatinine will also be measured approximately 24 hours post-dose (Day 2). eGFR will be estimated based on the equation provided in SAP Section 8.4.

Hematology will be performed at screening, at check-in on Day -1, and at study exit. The following will be assessed: complete blood count with differential, hemoglobin, and hematocrit.

Coagulation tests including prothrombin time (PT), activated partial thromboplastin time (aPTT) and international normalized ratio (INR) will be performed at screening, at check-in on Day -1 and at study exit. The following will be assessed: PT/INR and aPTT.

Urinalysis will be performed at screening, at check-in on Day -1, at approximately 24 and 72 hours post-dose, and at study exit. The following will be assessed: macroscopic examination, pH, specific gravity, protein, glucose, ketones, bilirubin, occult blood, nitrite, urobilinogen, and leukocytes. Unless otherwise specified, microscopic examination will be performed on abnormal findings and these results will be listed only.

Urine samples collected for PK assessment from dosing to 24 hours post-dose will also be used for analysis of creatinine, in order to calculate creatinine clearance (corrected for BSA), along with the serum creatinine value measured 24 hours post-dose. The total amount of creatinine excreted over 24 hours (addition of the 0-3, 3-6, 6-9, 9-12, 12-18, 18-24-hour post-dose time intervals) and the total volume of urine excreted over this 24-hour period will allow calculating creatinine clearance over 24 hours using the following formula:

$$\text{Corrected CrCl} = \frac{\text{Ucr}}{\text{Scr}} \times \frac{\text{Uvol}}{\text{Time}} \times \frac{1.73}{\text{BSA}}$$ where:

Corrected CrCl is expressed as mL/min/1.73 m^2 of BSA
Ucr is urine creatinine expressed in mg/dL
Uvol is the volume of urine expressed in mL
Scr is serum creatinine expressed in mg/dL
Time is expressed in minutes
BSA is calculated based on the height measured at screening and body Weight measured on Day -1, and expressed in m^2.

Urine samples for early markers of renal injury (KIM-1 and clusterin) and for creatinine will be collected prior to dosing, at approximately 12, 24, 36, and 48 hours post-dose, as well as on Day 8.

Listings of all clinical laboratory results, including those unscheduled, will be provided with the abnormal values flagged with "L" for low and "H" for high for continuous parameters, and “A” for abnormal for categorical parameters.

Descriptive statistics (mean, median, SD, Min, Max, and sample size) for each clinical laboratory test (continuous variables) will be presented by group and overall for screening, check-in on Day -1 and study exit. Unscheduled results will not be included in the summary tables except for baseline calculation if applicable. Change from baseline descriptive statistics for study exit will be presented. Baseline will be defined as the last results (scheduled or unscheduled) obtained prior to the administration of ELX-02. For categorical variables (urinalysis tests), the number of subjects (frequency and percentage) will be tabulated for each individual result (e.g., negative, positive, trace). Results from repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

A summary table of shifts from baseline to study exit measurements will be provided. Baseline will be defined in the same manner as described in the preceding paragraph for continuous variables. The shift tables will include normal, low, and high relative to the laboratory reference ranges (or normal-abnormal for categorical variables). Results from repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

9.4 Vital Signs

Blood pressure, respiratory rate, heart rate and oral temperature will be measured in a sitting position after at least 5 minutes of sitting (except for safety reasons) at screening, at check-out on Day 4 and at study exit. Blood pressure, heart rate, and oral temperature will also be measured prior to dosing and approximately 2, 4, 24 and 48 hours post-dose.

Descriptive statistics (mean, median, SD, Min, Max, and sample size) will be presented by group and overall for screening, 2, 4, 24 and 48 hours post-dose and study exit for each vital sign measurement. Baseline will be defined as the last results (scheduled or unscheduled) obtained prior to the administration of ELX-02. Unscheduled results will not be included in the summary tables except for baseline calculation if applicable. Change from baseline descriptive statistics for post-dose measurements as well as for study exit will be presented. Results from repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

A listing of all vital signs results will be provided by subject.

9.5 Electrocardiogram

Supine ECG will be performed at screening, prior to dosing, approximately 2 hours post-dose, at check-out on Day 4 and at study exit. The quantitative ECG measurements are heart rate (HR), PR interval, QRS interval, QT interval, QTcB (Bazett's formula correction) and QTcF (Fridericia formula correction).

Descriptive statistics (mean, median, SD, Min, Max, and sample size) will be presented by group and overall for screening, pre-dose, 2 hours post-dose at check-out on Day 4 and study exit for each ECG measurement. Baseline will be defined as the last results (scheduled or unscheduled) obtained prior to the administration of ELX-02. Unscheduled results will not be included in the summary tables except for baseline calculation if applicable. Change from baseline descriptive statistics for post-dose measurements as well as for study exit will be presented. Results from repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

A listing of all ECG results will be provided with the abnormal values flagged. In addition, a listing of significant ECG findings will be provided.

9.6 Injection Site Evaluation

Local reactions at the injection site will be evaluated by a trained observer and recorded: prior to study drug injection, approximately 0.75, 2, 6, 12, 24, 36, 48, and 72 hours post-dose, and at study exit. The severity of each local reaction at the injection site will be graded according to the NIH Division of AIDS (DAIDS) criteria presented in Table 9-1.

Table 9-1 Site Reactions to Injections and Infusions DAIDS Grading Table

Parameter	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Injection Site Pain or Tenderness	Pain or tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain or tenderness causing inability to perform usual social and functional activities	Pain or tenderness causing inability to perform basic self-care function OR hospitalization indicated
Injection site Erythema or Redness	2.5 to <5 cm in diameter OR 6.25 to <25 cm2 surface area AND symptoms causing no or minimal interference with usual social and functional activities	≥5 to <10 cm in diameter OR ≥25 to <100 cm2 surface area OR Symptoms causing greater than minimal interference with usual social and functional activities	≥10 cm in diameter OR ≥100 cm2 surface area OR ulceration OR secondary infection OR phlebitis OR sterile abscess OR drainage OR symptoms causing inability to perform usual social and functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper
Injection site Induration or Swelling	to <5 cm in diameter OR 6.25 to <25 cm2 surface area AND symptoms causing no or minimal interference with usual social and functional activities	≥5 to <10 cm in diameter OR ≥25 to <100 cm2 surface area OR Symptoms causing greater than minimal interference with usual social and functional activities	≥10 cm in diameter OR ≥100 cm2 surface area OR ulceration OR secondary infection OR phlebitis OR sterile abscess OR drainage OR symptoms causing inability to perform usual social and functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection site Pruritis	Itching localized to the injection site that is relieved spontaneously or in <48 hours of treatment	Itching beyond the injection site that is not generalized OR itching localized to the injection site requiring ≥48 hours treatment	Generalized itching causing inability to perform usual social and functional activities	NA

The number of subjects (frequency and percentage) will be tabulated for each individual parameter and reaction grading by time point of collection.

10. Pharmacokinetic Analyses

10.1 Handling of the Below the Lower Limit of Quantification (BLQ) and the No Reportable Concentration Values

When handling the below the limit of quantification (BLQ) data the following rules will apply for the derivation of PK parameters:

- Concentration values below the assay's limit of quantification (BLQ) for pre-dose samples will be treated as zero.
- Post-dose BLQ values prior to the first quantifiable concentration will be treated as zero.
- Post-dose BLQ values after the first quantifiable time point that are not followed by measurable concentration will be set to missing.
- The sampling times relative to dosing for pre-dose samples will also be treated as zero.

10.2 Handling of the Difference between the Scheduled and the Actual Sampling Times

The actual clock time for dosing and the actual clock time for each collection time for the PK samples will be recorded using electronic data capture. For all sampling times, the actual sampling times will be calculated as the difference between the sample collection actual clock time and the actual clock time of dosing. The actual post-dose sampling times expressed in hours and rounded off to three decimal digits will be used to calculate the PK parameters, except for pre-dose samples occurring prior to dosing, which will always be reported as zero (0.000), regardless of the time difference. Scheduled sampling times will be presented in concentration tables and mean graphs, while actual sampling times will be presented in the individual graphs in the PK section of the report. A listing of the actual times for PKs will be provided for PK samples.

10.3 Pharmacokinetic Parameters

10.3.1 Plasma Pharmacokinetic Parameters

For Group 1 to 4, a total of 13 blood samples will be drawn from each subject for quantitation of ELX-02 for pharmacokinetic analyses: 0.250, 0.500, 0.750, 1.00, 2.00, 4.00, 6.00, 12.0, 24.0, 36.0, 48.0, 72.0, and 168 hours post-dose.

Plasma ELX-02 concentrations data will be used to calculate the following parameters by standard non-compartmental methods:

AUC_{0-24}: Area under the concentration-time curve from time zero to 24 hours post-dose. Whenever concentration data is missing for the 24-hour post-dose sample for a subject, calculation of the AUC_{0-24} must not be done.

AUC_{0-72}: Area under the concentration-time curve from time zero to 72 hours post-dose. Whenever concentration data is missing for the 72-hour post-dose sample for a subject, calculation of the AUC_{0-72} must not be done.

AUC_{0-t}: Area under the concentration-time curve from time zero to the last non-zero concentration, calculated using the linear trapezoidal method.

AUC_{0-inf}: Area under the concentration-time curve from time zero to infinity (extrapolated), calculated as $AUC_{0-t}+C_t/K_{el,}$ where: C_t = the last observed non-zero concentration.

C_{max}:	Maximum observed concentration.
Residual area	Residual area, calculated as 100*(1- AUC_{0-t} / AUC_{0-inf}).
T_{max}:	Time of observed C_{max}.
$T_{½\ el}$:	Elimination half-life, calculated as log(2)/K_{el} using the natural logarithm.
K_{el}:	Elimination rate constant. This parameter will be the negative of the estimated slope of the linear regression of the log-transformed concentration (natural logarithm) versus time profile in the terminal elimination phase. At least 3 concentration points will be used in estimating K_{el}. The time point where log-linear K_{el} calculation begins ($K_{el\ Lower}$), and the actual sampling time of the last quantifiable concentration used to estimate the K_{el} ($K_{el\ Upper}$) will be reported with the correlation coefficient from the linear regression to calculate K_{el}.
CL/F:	Apparent total body clearance, calculated as Dose/AUC_{0-inf}.
V_d/F:	Apparent volume of distribution, calculated as Dose/(K_{el} x AUC_{0-inf}).

10.3.2 Urine Pharmacokinetic Parameters

For Group 1 to 4, Urine samples for PK analysis will be collected for each subject at the following time or time intervals: pre-dose (first void in the morning of Day 1), 0-3, 3-6, 6-9, 9-12, 12-18, 18-24, 24-36, 36-48, and 48-72 hours post-dose.

Urine concentrations from ELX-02 will be used to calculate the following parameters by standard non-compartmental methods:

$Ae_{interval}$:	Amount of drug excreted in urine for each time interval, calculated as the urine concentration multiplied by the urine volume.
Ae_{0-t}:	Cumulative urinary excretion from time zero to time t, calculated as the sum of the amounts excreted over each collection interval.
R_{max}:	Maximum rate of urinary excretion, calculated by dividing the amount of drug excreted in each collection interval by the time over which it was collected.
T_{Rmax}:	Time of maximal urinary excretion, calculated as the midpoint of the collection interval during which R_{max} occurred.
Fe_{0-t}:	Fraction (% dose) excreted unchanged, calculated as A_e/D_{sc}.
CL_R:	Renal clearance, calculated as Ae_{0-24} / AUC_{0-24}.

Additional pharmacokinetic analysis may be performed. Upon the Sponsor's request, pharmacokinetic repeats might be performed according to inVentiv's SOP. If re-assays are requested for pharmacokinetic reasons, final results will be presented using re-assay values, while results with original values will be presented in an appendix of the report as supportive data.

Some PK parameters may not be calculated for all or some subjects, at the discretion of the inVentiv pharmacokineticist, if the concentration data is not deemed to be amenable to evaluation. Explanations for PK parameters that could not be estimated will be provided in the PK report. Additional exploratory pharmacokinetic parameters such as urine half-life may also be assessed.

10.4 Statistical Analyses

Renal function assessment will be reported with the method using MDRD4 equation with serum creatinine concentration. GFR estimated with serum creatinine concentrations will be used to classify subjects and this classification will serve for primary analyses.

Individual and mean (±SD) plasma concentration versus time curves will be presented for both linear and semi-log scales. Descriptive statistics (arithmetic and geometric means, standard deviation [SD], coefficient of variation [CV%], minimum [Min], maximum [Max], and median) of the plasma concentrations versus time will be presented as well for the PK parameters according to renal function groups included in the study (Mild, Moderate, Severe renal impairment, and Control). A graphical description of the relationship between renal function measures and PK will be presented.

For plasma PK parameters, using GLM procedures in SAS, ANOVA will be performed on untransformed T_{max}, K_{el} and $T_{½\ el}$ and on ln-transformed AUC_{0-24}, AUC_{0-72}, AUC_{0-t}, AUC_{0-inf}, and C_{max}. Factors incorporated in the model will include Group as a fixed effect. Each statistical comparison (i.e.: Mild vs. Control, Moderate vs. Control, and Severe vs. Control) will be done separately.

Sample code for the procedure in SAS® codes for ANOVA (Mild vs. Control) is specified below:

```
proc glm data=basepk;
  where GROUP in ('1','4');
  class GROUP;
  model VAR = GROUP / ss3;
  means GROUP;
  lsmeans GROUP / cl alpha=0.1 pdiff;
 estimate 'Mild vs. Control' GROUP 1 -1;
run;
```

The ratios of geometric means (Mild/Control, Moderate/Control, Severe/Control) and their associated 90% CI will be calculated according to ANOVA results for AUC_{0-24}, AUC_{0-72}, AUC_{0-t}, AUC_{0-inf}, and C_{max}. Inter-subject Coefficient of variation will be estimated. Two-sided p-values will also be derived as exploratory analyses to provide statistical inferences at the alpha 5% level of significance.

Derived calculations obtained from the ANOVA analyses will be performed as per the following:

- Inter Subject CV = 100 * SQRT ($e^{[MSE]}$ - 1);

- Ratio = $100 * e^{DIFFERENCE}$, where DIFFERENCE is the point estimate of renal function groups difference (e.g. Mild – Control, Mild – Control, and Severe - Control) on the ln-transformed scale; and

- 90% Confidence Limits = $100 * e^{(DIFFERENCE \pm t_{(dfResidual)} * SE_{DIFFERENCE})}$.

T_{max} will be analyzed nonparametrically (using the Hodges-Lehmann method) with point estimates and 90% CIs for the median differences of Tmax between groups (Mild-Control, Moderate-Control, Severe-Control).

```
proc npar1way hl alpha=.10 data= basepk;
    class GROUP;
    var Tmax;
    exact hl;
    ods select WilcoxonScores HodgesLehmann;
  run;
```

For urine PK parameters, Ae_{0-t} and R_{max} will be analysed in the same manner as plasma PK parameters (AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, and C_{max}).

In the event that renal impairment has a clinically relevant effect on ELX-02 PK, the relationship between renal function and appropriate PK parameters for ELX-02 (e.g., CL/F, AUC_{0-24}, AUC_{0-72}, AUC_{0-t}, AUC_{0-inf}, and C_{max}) will be determined by a linear or non-linear regression.

Study results including the graphical description and the relationships between renal function and relevant pharmacokinetic parameters will be used to elaborate specific dosing recommendations if deemed clinically relevant.

Additional and exploratory statistical analysis may be performed.

11. Percentages and Decimal Places

If not otherwise specified, the following rules will be applied, with the exception of PK tables and listings described below:

- Percentages will be presented to one decimal point.
- Percentages equal to 0 or 100 will be presented as such without a decimal point.
- Minimum and maximum will be presented with the same precision as the original values and, mean, standard deviation, and median will be presented with one more decimal place than the original values.

All digits will be used for pharmacokinetic and statistical PK calculations. For PK tables and listings, the final reportable results or data will be presented by rounding off to two decimal digits, except for the following situations (this applies to individual data and descriptive statistics):

- K_{el} and correlation (Corr.) data: rounded off to four decimal digits.
- Pharmacokinetic parameters related to time such as T_{max}, $K_{el\ Lower}$, and $K_{el\ Upper}$ must be reported with the same precision as the actual sampling time: rounded off to 3 decimal digits.
- Concentration versus time data, as well as C_{max}: reported as they appear in corresponding dataset.
- Ratios and 90% confidence intervals, and inter-CV (%) will be presented to two decimal places.

12. Data Handling

The ELX-02 plasma and urine concentrations, safety and tolerability data will be received as SAS® datasets from the inVentiv data management facility. Screening failures and ineligible volunteer's data (subject disposition) will be received from the clinical site as source data.

13. Handling of Missing Data

For PK, only observed data will be used in the data analysis except for concentration values BLQ and samples with no reportable value occurring prior to dosing as described in Section 10.1. No attempt will be made to extrapolate or interpolate estimates for missing data.

For safety,

- If an AE is recorded with an onset date corresponding to a dosing day, but the time is missing, then the AE will be assigned to the treatment as a TEAE.
- If an AE is recorded with an onset date that does not correspond to the dosing day, but the time is missing, then the AE will be assigned to the treatment as a TEAE if AE onset date is after dosing date.
- If an AE is recorded with an onset date where day and time are both missing, then the AE allocation to the treatment will be done on a case by case basis considering available information (e.g. AE end date, AE comments, subject disposition).

14. Software to be Used

PK analysis will be performed using Phoenix WinNonlin® version 8 or higher, which is validated for bioequivalence/bioavailability studies by inVentiv. The safety data tables and listings, as well as PK tables and listings will be created using SAS®, release 9.2 or a higher version. PK figures will be created using R (version 3.5). The PK report text will be created using Microsoft® Office Word 2010, or a higher version.

15. Reference List

None.