PROTOCOL 182023
Eloxx Pharmaceuticals Study Number: EL-008

IND Number: 137391

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

Contract Research Organization:
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Protocol Historical File

Version number	Brief description/summary of changes	Date
Final 2.0	Version submitted to the IEC.	06-NOV-2018

Signature Page

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Signature Page for Site 01

Division of Clinical Pharmacology, University of Miami

[redacted]

Principal Investigator:

I have carefully read this study protocol and agree that it contains all necessary information required to conduct this study. I agree to conduct the study according to this protocol (including any amendments) and in accordance with Division of Clinical Pharmacology of University of Miami's Standard Operating Procedures (SOPs), ICH Good Clinical Practices (GCP), and all other applicable regulations.

[redacted]

Signature Page for Site 02

inVentiv Health Clinical Research Services LLC, a Syneos Health company

[redacted]

Principal Investigator:

I have carefully read this study protocol and agree that it contains all necessary information required to conduct this study. I agree to conduct the study according to this protocol (including any amendments) and in accordance with inVentiv's SOPs, ICH GCP, and all other applicable regulations.

[redacted]

1. Facilities and Responsible Staff

1.1 Contract Research Organization

[redacted]

1.2 Clinical Research Facilities

This study will be conducted at the following facilities:

Site 01:

[redacted]

Site 02:

[redacted]

1.3 Biomedical Laboratory Facilities

Biomedical laboratory testing will be performed by the following laboratories:

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

If another biomedical laboratory is used, this will be documented and annexed to the protocol.

1.4 Clinical Pharmacology

[redacted]

Data Management, Pharmacokinetics and Statistical Analyses:

[redacted]

Protocol Writing:

[redacted]

1.5 Bioanalytical Facility

[redacted]

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2. Synopsis of Protocol

Project No.:	182023 Eloxx Pharmaceuticals study number: EL-008
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
Study Drug:	ELX-02 [6'-(R)-Methyl-5-O-(5-amino-5,6-dideoxy-α-L-talofuranosyl)-paromamine sulfate]
Study Phase and Type:	Phase 1 – Pharmacokinetics in Patients with Impaired Renal Function
Objectives:	Primary objective: To determine the effect of various severities of renal impairment 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. 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.
Study 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. Subjects will be categorized in 4 groups: Group 1: subjects with mild renal impairment Group 2: subjects with moderate renal impairment Group 3: subjects with severe renal impairment Group 4 (control group): subjects with normal renal function The mild (Group 1) and moderate (Group 2) patients with renal disease will be dosed first, in a parallel fashion. At this point, interim PK analyses will be performed and a safety committee composed of Sponsor and Contract Research Organization (CRO) members will jointly review the PK data before dosing the patients with severe renal disease (Group 3). Control subjects (Group 4) will be recruited after the recruitment of Groups 1 to 3.
Subjects:	It is targeted to enroll approximately 24 to 26 adult male or female subjects, ≥ 18 and ≤ 80 years of age. Subjects with normal renal function will be non-smoker while renally impaired (RI) subjects may be light smoker (no more than 5 cigarettes/day or equivalent) or non-smoker. An effort will be made to carefully match the healthy subjects in the control group with the RI subjects by age (± 10 years), body mass index [BMI] (±15%), and gender, to the extent possible. Attempts will be made to enroll at least two subjects of each gender in each group and subjects with various BMIs. A mean matching procedure will be performed. 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). Subjects who withdraw or are withdrawn from the study after dosing, for reasons other than safety and tolerability, may be replaced in order to ensure a minimum of 6 completed subjects per group. The total number of subjects dosed (including potential replacement subjects) will remain within a maximum of 8 subjects per group and within a maximum of 32 subjects for the whole

	study.
Inclusion and Exclusion Criteria for Subjects With Renal Impairment (Groups 1 to 3):	Inclusion Criteria: 1) Male or female, non-smoker and/or light smoker (up to 5 cigarettes or equivalent/day), ≥ 18 and ≤ 80 years of age, with BMI ≥ 18.0 and ≤ 40.0 kg/m^2 and body weight ≥ 50.0 kg for males and ≥ 45.0 kg for females. 2) Have a diagnosis of renal impairment that has been stable, without any significant change in overall disease status in the last 3 months prior to screening as determined by the Principal Investigator (PI). 3) Have an estimated glomerular filtration rate (eGFR) expressed in mL/min/1.73 m^2 (Modification of Diet in Renal Disease 4-variable [MDRD4] equation) at screening within the range of: a) Group 1 - Mild Group: 60 - 89 mL/min/1.73 m^2; b) Group 2 - Moderate Group: 30 - 59 mL/min/1.73 m^2; c) Group 3 - Severe Group: < 30 mL/min/1.73 m^2 not requiring dialysis. eGFR results that are deemed inconsistent with the usual stage of renal impairment may be repeated. Subjects are categorized into severity group at screening. If the eGFR scores change on Day-1 or other visit due to a non-clinically significant change in clinical status or laboratory result, the subject keeps the original severity group. 4) Subject may have stable treated medical illnesses and underlying diseases producing the renal impairment such as diabetes, hypertension, or cardiovascular disease, providing that, in the opinion of the PI, the disease is stable. 5) Have normal or non-clinically significant findings at physical examination, vital signs and electrocardiogram (ECG) and normal limits or non-clinically significant deviations in clinical laboratory evaluations at screening, with the exception of findings that in the opinion of the PI are consistent with subject's renal impairment or due to other stable diseases. Clinical data, ECG and laboratory tests may be repeated at the discretion of the Investigator. 6) Other than renal impairment, have no other conditions which may significantly impact study drug absorption or metabolism, as determined by the PI. 7) Stable medical regimen, deemed not to interact with study drug PK, for 14 days prior to dosing, except for routine daily management of electrolytes (e.g. potassium), acid-base, or other associated disorders expected in patients with renal impairment. The approval of the medications for the management of renal impairment and the treatment of concomitant stable medical conditions (e.g. diabetes, hypertension, associated stable cardiovascular disease, stable psychiatric conditions) is at the discretion of the PI. 8) Females of childbearing potential who are sexually active with a non-sterile male partner (sterile male partners are defined as men vasectomized since at least 6 months) must be willing to use one of the following acceptable contraceptive method throughout the study and for 30 days after study drug administration: a) simultaneous use of intra-uterine contraceptive device, without hormone release system, placed at least 4 weeks prior to study drug administration, and condom for the male partner;

	b) simultaneous use of diaphragm with intravaginally applied spermicide and male condom for the male partner, starting at least 21 days prior to study drug administration. 9) Male subjects who are not vasectomized for at least 6 months, and who are sexually active with a non-sterile female partner (sterile female partners include post-menopausal females and surgically sterile females) must be willing to use one of the following acceptable contraceptive method from dosing until at least 90 days after study drug administration: a) simultaneous use of a male condom and, for the female partner, hormonal contraceptives (used since at least 4 weeks) or intra-uterine contraceptive device (placed since at least 4 weeks); b) simultaneous use of a male condom and, for the female partner, a diaphragm with intravaginally applied spermicide. 10) Male subjects (including men who have had vasectomy) with a pregnant partner must agree to use a condom from dosing until at least 90 days after study drug administration. 11) Male subjects must be willing not to donate sperm until 90 days following study drug administration. 12) Able to understand and willing to sign the Informed Consent Form (ICF) and comply with the study restrictions. <u>Exclusion Criteria</u>: 1) Unstable renal function or acute exacerbation of renal disease within 14 days of study drug administration, as indicated by recent history or worsening of clinical and/or laboratory signs of renal impairment as judged by the PI. For inclusion and for categorization by MDRD4 or other equations, laboratory results that are deemed inconsistent with the usual stage of renal impairment may be repeated. 2) Has a functioning renal transplant. 3) Major illness or surgery within 4 weeks prior to dosing. 4) Clinically significant unstable medical condition or history of any illness that may increase the risk associated with study participation or investigational drug administration or may interfere with the interpretation of study results and, in the judgment of the PI, would make the subject inappropriate for entry into this study. Subjects who do not qualify based on a reversible medical condition or mild inter-current illness may be re-evaluated after further testing/examination or re-screened after the condition is resolved. 5) Positive test for hepatitis B, hepatitis C, or human immunodeficiency virus (HIV) at screening. 6) History of allergic reactions, hypersensitivity or toxic reactions to aminoglycosides. 7) History of anaphylaxis. 8) Supine 12-lead ECG abnormalities at screening considered clinically significant by the PI. 9) Clinically significant vital sign abnormalities (systolic blood pressure lower than 90 or over 160 mmHg, diastolic blood pressure lower than 40 or over 100 mmHg, or heart rate less than 45 or over 100 bpm) at screening. 10) History of significant drug or alcohol abuse within six months prior to screening. 11) Participation in a clinical research study involving the administration of an

	investigational or marketed drug or device within 30 days (or 5 half-lives, whichever is longer) prior to dosing, administration of a biological product in the context of a clinical research study within 90 days prior to dosing, or concomitant participation in an investigational study involving no drug or device administration. 12) Positive urine drug screen or alcohol test at screening, unless the positive drug screen is due to prescription drug use that is documented and approved by the PI. 13) Female subject with positive pregnancy test at screening. 14) Breast-feeding or pregnant subject within 6 months prior to study drug administration. 15) Use of any drugs known as strong inducer or inhibitor of hepatic drug metabolism within 30 days prior to study drug administration. 16) Use of medication other than stable medications approved by the PI and topical products without significant systemic absorption: a) any new prescription medication within 14 days prior to dosing; b) over-the-counter (OTC) products or natural health products (including herbal remedies such as St. John's wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) within 7 days prior to dosing, with the exception of the occasional use of acetaminophen (that is allowed up to 2 g daily), unless the products are used by RI subjects as part of their routine care; c) a depot injection or an implant of any drug within 3 months prior to dosing. 17) The following medications are prohibited during the study: neuromuscular blocking agents (e.g. succinylcholine, tubocurarine), neurotoxic and/or nephrotoxic drugs (e.g. other aminoglycosides, cisplatin, cephaloridine, polymyxin B, vancomycin, viomycin). At the discretion of the PI, a suitable substitute medication may be prescribed to replace the prohibited medication. 18) Donation of plasma within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to the first dosing. 19) Any reason which, in the opinion of the PI, would prevent the subject from participating in the study. 20) Inability to be venipunctured and/or tolerate catheter venous access. 21) Presence of mitochondrial mutation(s) making the subject susceptible to aminoglycoside toxicity. 22) Presence of signs of dehydration, recent history of neuromuscular blockade or clinically significant history of vestibular impairment.
Inclusion and Exclusion Criteria for Subjects with Normal Renal Function (Group 4):	Inclusion Criteria: 1) Male or female, non-smoker (no use of tobacco or nicotine products within 3 months prior to screening), ≥ 18 and ≤ 80 years of age, with BMI ≥ 18.0 and ≤ 40.0 kg/m^2 and body weight ≥ 50.0 kg for males and ≥ 45.0 kg for females. 2) Have an eGFR ≥ 90 mL/min/1.73 m^2 (MDRD4 equation).

	3) Healthy as defined by: a) the absence of clinically significant illness and surgery within 4 weeks prior to dosing. Subjects vomiting within 24 hours pre-dose will be carefully evaluated for upcoming illness/disease. Inclusion pre-dosing is at the discretion of the PI. b) the absence of clinically significant history of hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, and immunologic disease. 4) Matched to subjects with RI (mild, moderate or severe) according to gender, age (± 10 years), and BMI (± 15%). 5) Females of childbearing potential who are sexually active with a non-sterile male partner (sterile male partners are defined as men vasectomized since at least 6 months) must be willing to use one of the following acceptable contraceptive method throughout the study and for 30 days after study drug administration: a) simultaneous use of intra-uterine contraceptive device, without hormone release system, placed at least 4 weeks prior to study drug administration, and condom for the male partner; b) simultaneous use of diaphragm with intravaginally applied spermicide and male condom for the male partner, starting at least 21 days prior to study drug administration. 6) Male subjects who are not vasectomized for at least 6 months, and who are sexually active with a non-sterile female partner (sterile female partners include post-menopausal females and surgically sterile females) must be willing to use one of the following acceptable contraceptive method from dosing until at least 90 days after study drug administration: a) simultaneous use of a male condom and, for the female partner, hormonal contraceptives (used since at least 4 weeks) or intra-uterine contraceptive device (placed since at least 4 weeks); b) simultaneous use of a male condom and, for the female partner, a diaphragm with intravaginally applied spermicide. 7) Male subjects (including men who have had vasectomy) with a pregnant partner must agree to use a condom from the first dosing until at least 90 days after study drug administration. 8) Male subjects must be willing not to donate sperm until 90 days following study drug administration. 9) Able to understand and willing to sign the ICF and comply with the study restrictions. <u>Exclusion Criteria</u> 1) Any clinically significant abnormality at physical examination or clinically significant abnormal laboratory test results at screening. 2) Positive test for hepatitis B, hepatitis C, or HIV at screening. 3) History of allergic reactions, hypersensitivity or toxic reactions to aminoglycosides. 4) Evidence or history of clinically relevant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, immunologic, or allergic disease. This includes any acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational drug administration or may interfere with the interpretation of study results

	and, in the judgment of the PI, would make the subject inappropriate for entry into this study. 5) History of anaphylaxis. 6) Clinically significant supine 12-lead ECG abnormalities at screening, e.g., QTcF >450 msec for men and >470 msec for women, or a QRS interval >120 msec. 7) Clinically significant vital sign abnormalities (systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 50 or over 90 mmHg, or heart rate less than 50 or over 100 bpm) at screening. 8) History of significant alcohol abuse within one year prior to screening or regular use of alcohol within six months prior to the screening visit (more than fourteen units of alcohol per week [1 unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol]). 9) History of significant drug abuse within one year prior to screening or use of soft drugs (such as marijuana) within 3 months prior to the screening visit or hard drugs (such as cocaine, phencyclidine [PCP], crack, opioid derivatives including heroin, and amphetamine derivatives) within 1 year prior to screening. 10) Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days (or 5 half-lives, whichever is longer) prior to dosing, administration of a biological product in the context of a clinical research study within 90 days prior to dosing, or concomitant participation in an investigational study involving no drug or device administration. 11) Positive urine drug screen, alcohol test, or urine cotinine test at screening. 12) Female subject with positive pregnancy test at screening. 13) Breast-feeding or pregnant subject within 6 months prior to study drug administration. 14) Use of any drugs known to induce or inhibit hepatic drug metabolism within 30 days prior to study drug administration. 15) Use of medication other than topical products without significant systemic absorption: a) prescription medication within 14 days prior to dosing; b) OTC products and natural health products (including herbal remedies such as St. John's wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) within 7 days prior to dosing, with the exception of the occasional use of acetaminophen (that is allowed up to 2 g daily); c) a depot injection or an implant of any drug within 3 months prior to dosing. 16) Donation of plasma within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to dosing. 17) Hemoglobin < 12.8 g/dL (males) and < 11.5 g/dL (females) and hematocrit < 37% (males) and < 32% (females) at screening. 18) Any reason which, in the opinion of the PI, would prevent the subject from participating in the study. 19) Inability to be venipunctured and/or tolerate catheter venous access.

<table>
<tr><td></td><td>20) Presence of mitochondrial mutation(s) making the subject susceptible to aminoglycoside toxicity.
21) Presence of signs of dehydration, recent history of neuromuscular blockade or clinically significant history of vestibular impairment.</td></tr>
<tr><td>Screening Procedures:</td><td>Demographic data, medical and medication histories, complete physical examination, body measurements, 12-lead ECG, vital signs (blood pressure [BP], heart rate [HR], respiratory rate [RR] and oral temperature [OT]), hematology, biochemistry, coagulation, serum cystatin C measurement, HIV, hepatitis B and C tests, urinalysis, genetic testing for mitochondrial mutations, urine drug screen, alcohol test (breath or urine), urine pregnancy test, and urine cotinine test (for Group 4 only).</td></tr>
<tr><td>Renal Function Assessment at Screening:</td><td>Renal function will be assessed at screening using the MDRD4 equation for the estimation of the glomerular filtration rate (eGFR). Subjects eligibility will be based on screening results and subjects will be assigned at check-in to one of the following groups:
<table>
<tr><th>Group</th><th>Description</th><th>eGFR (mL/min/1.73m^2)</th><th>Number of Subjects</th></tr>
<tr><td>1</td><td>Mild decrease in GFR</td><td>60-89</td><td>6</td></tr>
<tr><td>2</td><td>Moderate decrease in GFR</td><td>30-59</td><td>6</td></tr>
<tr><td>3</td><td>Severe decrease in GFR</td><td>< 30; not requiring dialysis</td><td>6</td></tr>
<tr><td>4</td><td>Control (normal) GFR</td><td>≥ 90</td><td>6-8</td></tr>
</table>
<u>MDRD4 (4-variable) equation</u>:
eGFR = 175 x (Standardized Scr)$^{-1.154}$ x (Age)$^{-0.203}$ x (0.742 if female) x (1.212 if African American) where:
GFR is expressed as mL/min/1.73 m^2 of body surface area (BSA)
Scr is serum creatinine expressed in mg/dL
Age is expressed in years.
Variables are: serum creatinine, age, gender, and race.</td></tr>
<tr><td>In-Patient Stay and Visit:</td><td>Subjects will remain as in-patients from the morning of Day -1 until after the 72-hour post-dose blood draw. Subjects will come back for a follow-up visit on Day 8 (±1 day).</td></tr>
<tr><td>Study Drug Administration:</td><td>Subjects will receive a single dose of 1 mg/kg ELX-02 on Day 1. ELX-02 will be administered as a SC injection to the abdominal region around the umbilicus.</td></tr>
<tr><td>Food and Fluid Intake:</td><td>For standardization purpose, ELX-02 will be administered at least 30 minutes after subjects have been served a light breakfast. No food will be allowed until at least 1 hour after dosing. Standardized meals will be served at appropriate times thereafter.
To keep subjects well hydrated, they will be asked to consume 240 mL of fluids within 120 minutes prior to dosing. Water will be provided ad libitum at all other times, however the study staff will encourage subjects to drink at least 200 mL of fluids at the beginning of each 12-hour post-dose period.</td></tr>
</table>

Study Restrictions:	Subjects will be asked to refrain from using products that may potentially affect their safety and/or the pharmacokinetic profile of the study drug. Main study restrictions include: - prescription medication (other than stable medications approved by the PI for Groups 1-3) from 14 days prior to dosing until study exit; - OTC products from 7 days prior to dosing until study exit, with the exception of the occasional use of acetaminophen (that is allowed up to 2 g daily) and products used by RI subjects as part of their routine care; - natural health products from 7 days pre-dose until study exit, except for products used by RI subjects as part of their routine care; - food containing poppy seeds within 24 hours prior to admission; - food or beverages containing xanthine derivatives or xanthine-related compounds or energy drinks from 48 hours pre-dose until after the 72-hour blood sample collection; - food or beverages containing grapefruit, Seville orange, starfruit, pomegranate, pineapple, or pomelo from 7 days pre-dose until after the 72-hour blood sample collection; - alcohol-based products from 24 hours prior to admission until after the 72-hour blood sample collection; - for light smoking subjects in Groups 1 to 3, smoking will be prohibited from at least 2 hours pre-dose until 2 hours post-dose and a maximum of 5 cigarettes per day will be allowed while subjects are in-patients in the clinic. Subjects will be allowed to engage in normal activity but will avoid lying down or sleeping, unless medically necessary or procedurally required, for 4 hours after drug administration. <u>For subjects in Groups 1 to 3:</u> Short outings will be permitted during the in-patient period for smoking subjects. Non-smokers could also go out for supervised outings at the discretion of the site staff. Outings will be supervised at all times by the clinical staff to ensure compliance with protocol and will be limited to the grounds surrounding the site, as per the clinical site specific procedures for supervised outings.
Blood Sample Collection for PK Analysis:	A total of 13 blood samples will be collected for PK analysis for each subject of Groups 1 to 4: 0.25, 0.5, 0.75, 1, 2, 4, 6, 12, 24, 36, 48, 72, and 168 (Day 8) hours post-dose.
Urine Sample Collection for PK Analysis:	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 Sample Collection for Creatinine Analysis:	Urine samples collected for PK assessment from dosing to 24 hours post-dose will also be used for analysis of creatinine to calculate creatinine clearance (ClCr). Urine sample for creatinine analysis will be collected along with samples for renal injury biomarkers: prior to dosing and at approximately 12, 24, 36, and 48 hours post-dose, and on Day 8.
Renal Injury Biomarkers:	Urine samples for early markers of renal injury (KIM-1 and clusterin) will be collected prior to dosing and at approximately 12, 24, 36, and 48 hours post-dose, and on Day 8.

On-Study Safety Procedures:	Physical examination and body measurements: Brief physical examination: on Day -1 and at check-out on Day 4. Body weight measurement: on Day -1. Laboratory tests: Serum pregnancy test, alcohol test (breath or urine), urine cotinine test (for Group 4 only), and urine drug screen: on Day -1. Biochemistry, hematology, and coagulation (all clinical laboratory tests following a fasting period of at least 8 hours): on Day -1. Urinalysis: on Day -1 and at approximately 24 and 72 hours post-dose. Serum creatinine: approximately 24 hours post-dose. Vital signs: BP, HR and OT: before dosing and approximately 2, 4, 24, and 48 hours post-dose. BP, HR, RR and OT: at check-out on Day 4. ECG: 12-lead ECG: before dosing, approximately 2 hours post-dose and at check-out on Day 4. Injection site evaluation: Injection site evaluation: before dosing and approximately 0.75, 2, 6, 12, 24, 36, 48, and 72 hours post-dose. Medical surveillance and AE monitoring: Medical surveillance: Subjects will be monitored throughout the study by the site staff for adverse events (AEs). A physician will be on site/campus for drug administration and until 4 hours post-dose, and available on call for the remainder of the study.
Study Exit / Early Termination Procedures:	Study exit procedures are scheduled to be performed at the follow-up visit on Day 8 (±1 day) or within 14 days after the last participation of the subject in the study in case of early termination. The study exit procedures include: brief physical examination, hematology, biochemistry, coagulation, urinalysis, vital signs, 12-lead ECG, urine pregnancy test, injection site evaluation, and AE monitoring.
Analytical Methods:	QPS will analyze ELX-02 in plasma and urine samples using validated methods.
Pharmacokinetics:	Parameters calculated with plasma concentrations of ELX-02: AUC_{0-72}, AUC_{0-t}, AUC_{0-inf}, C_{max}, Residual area, T_{max}, $T_{½\ el}$, K_{el}, CL/F, and V_d/F. Parameters calculated with urine concentrations of ELX-02: $Ae_{interval}$, Ae_{0-t}, R_{max}, T_{Rmax}, Fe_{0-t}, and CL_R.
Statistical Analyses:	Pharmacokinetics: 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_{0-72}, AUC_{0-t}, AUC_{0-inf}, and C_{max} at the alpha level of 0.05. The ratio of geometric means (Mild/Control, Moderate/Control, Severe/Control) and 90% confidence interval (CI) for the ratio of geometric means, based on least-squares means from the ANOVA of the ln-transformed data, will be calculated for AUC_{0-72}, AUC_{0-t}, AUC_{0-inf}, and C_{max}. T_{max} will be analyzed nonparametrically with point estimates and 90% CIs for the median differences of T_{max} between treatments

	(Mild-Control, Moderate-Control, Severe-Control). For urine PK parameters, using GLM procedures in SAS, ANOVA will be performed on ln-transformed Ae_{0-t} and R_{max} at the alpha level of 0.05 to compare groups (Mild, Moderate, Severe and Control). The ratio of geometric means (Mild/Control, Moderate/Control, Severe/Control) and 90% CI for the ratio of geometric means, based on least-squares means from the ANOVA of the ln-transformed data, will be calculated for Ae_{0-t} and R_{max}. <u>Safety and tolerability:</u> Treatment-emergent AEs (TEAEs) will be tabulated by study group. Changes from baseline values in vital signs, ECG, and clinical laboratory parameters will be evaluated. Safety and tolerability data will be reported using descriptive statistics. Details of statistical analyses will be developed in a Statistical Analysis Plan (SAP).

3. List of Abbreviations

AE	Adverse Event
Ae	Cumulative Urinary Excretion
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BMI	Body Mass Index
BP	Blood Pressure
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
CI	Confidence Interval
CF	Cystic Fibrosis
CFR	Code of Federal Regulations
CFTR	Cystic Fibrosis Transmembrane Regulator
CL_R	Renal Clearance
CL/F	Total Body Clearance
ClCr	Creatinine Clearance
C_{max}	Maximum Plasma Concentration
CPK	Creatine Phosphokinase
CRF	Case Report Form
CRO	Contract Research Organization
CV	Coefficient of Variation
DAIDS	Division of AIDS
DMD	Duchenne Muscular Dystrophy
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
ERSG	Eukaryotic Ribosomal Specific Glycoside
FDA	Food and Drug Administration
Fe	Fraction of Drug Excreted Unchanged
GCP	Good Clinical Practices
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HBsAg	Hepatitis B
HCV	Hepatitis C
HEENT	Head, Eyes, Ears, Nose, and Throat
HIV	Human Immunodeficiency Virus
HR	Heart Rate
IB	Investigator's Brochure

ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
INR	International Normalized Ratio
IRB	Institutional Review Board
IV	Intravenous
K_{el}	Elimination Rate Constant
kg	Kilogram
L	Liters
MDMA	3,4-Methylenedioxymethamphetamine
MDRD4	Modification of Diet in Renal Disease 4-variable Equation
mg	Milligram
mL	Milliliter
mmHg	Millimeters of Mercury
MPS I	Mucopolysaccharidosis Type I
NOAEL	No Observed Adverse Event Level
OT	Oral Temperature
OTC	Over-the-Counter
PCP	Phencyclidine
PI	Principal Investigator
PK	Pharmacokinetic
aPTT	Activated Partial Thromboplastin Time
PT	Prothrombin Time
QA	Quality Assurance
QC	Quality Control
QRS	QRS Complex
QT	QT Interval
QTcF	Fridericia's Corrected QT interval
RI	Renally Impaired
R_{max}	Maximum Rate of Urinary Excretion
RR	Respiratory Rate
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS	Severe Acute Respiratory Syndrome
SC	Subcutaneous
SD	Standard Deviation
SOP	Standard Operation Procedure
SPVG	Syneos Health Safety and Pharmacovigilance
TEAE	Treatment-Emergent Adverse Event
$T_{1/2}$	Half-Life
$T_{½\ el}$	Elimination Half-Life

T_{max}	Time of Maximum Concentration
V_d/F	Apparent Volume of Distribution

4. Schedule of Events

Table 1. Schedule of Events for Study Groups 1 to 4

PROCEDURE	Screening	Groups 1 to 4					Study Exit (Follow-Up Visit on Day 8 ± 1) or Early Termination
		D-1	D1	D2	D3	D4	
Demographic Data	X						
Medical and Medication Histories	X						
Review and Monitoring of AEs and Concomitant Medications		X	X	X	X	X	X
Physical Examination[1]	X	X				X	X
Body Measurements	X	X[2]					
Vital Signs (BP, HR, RR, OT)	X		X[3]	X[3]	X[3]	X[3]	X
ECG	X		X[4]			X[4]	X
Hematology	X	X[5]					X
Biochemistry	X	X[5]		X[6]			X
Serum Cystatin C Measurement[7]	X						
Coagulation	X	X[5]					X
Urinalysis	X	X[5]		X		X	X
HIV and Hepatitis	X						
Genetic Testing for Mitochondrial Mutations	X						
Urine Drug Screen	X	X					
Urine Cotinine Test (for Group 4 only)	X	X					
Alcohol Test (Breath or Urine Test)	X	X					
Serum Pregnancy Test		X					
Urine Pregnancy Test	X						X
Injection Site Evaluation			X[8]	X[8]	X[8]	X[8]	X
In-Patient Stay		X	X	X	X		
Study Drug Administration			X				
PK Blood Samples[9]			X	X	X	X	X
PK Urine Sample[10]			X	X	X	X	
Urine Sample for Creatinine[11]			X	X	X		X
Renal Injury Biomarkers[12]			X	X	X		X

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

2 Body weight only on Day -1.

3 BP, HR and OT: before dosing and approximately 2, 4, 24, and 48 hours post-dose. BP, HR, RR and OT: at check-out on Day 4.

4 ECG: pre-dose, approximately 2 hours post-dose and at check-out on Day 4.

5 Laboratory assessments (i.e., biochemistry, hematology, coagulation, and urinalysis) will be done in the morning of Day -1 following a fasting period of at least 8 hours.

6 Serum creatinine only: approximately 24 hours post-dose.

7 Cystatin C will be measured at screening and cystatin GFR will be estimated for additional data analysis, only if needed. These values will have no impact on subject's eligibility and categorization.

8 Injection site evaluation: before dosing and approximately 0.75, 2, 6, 12, 24, 36, 48, and 72 hours post-dose.

9 PK blood samples: 0.25, 0.5, 0.75, 1, 2, 4, 6, 12, 24, 36, 48, 72, and 168 (Day 8) hours post-dose.

10 Urine samples for PK analysis: 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.

11 Urine samples collected for PK assessment from 0 to 24 hours post-dose will also be used for analysis of creatinine to calculate ClCr. Spot urine sample for creatinine analysis will be collected prior to dosing and at approximately 12, 24, 36, and 48 hours post-dose, and on Day 8.

12 Renal Injury Biomarkers (KIM-1 and clusterin): pre-dose, at approximately 12, 24, 36, and 48 hours post-dose and on Day 8.

5. Introduction

5.1 Background Information on ELX-02[1]

Eloxx Pharmaceuticals is developing ELX-02 [6'-(R)-Methyl-5-O-(5-amino-5,6-dideoxy-α-L-talofuranosyl)- paromamine sulfate], a small molecule, new chemical entity for SC administration. The target indication for ELX-02 is the treatment of genetic diseases caused by nonsense mutations.

ELX-02 is a [redacted] eukaryotic ribosomal specific glycoside (ERSG) optimized as a translational read-through drug. Chemically, ELX-02 can be synthesized from standard precursor blocks. Biologically, ELX-02 exhibits high selectivity towards the eukaryotic ribosome and decreased binding to mitochondrial and prokaryotic ribosomes. ELX-02 induces high read-through activity leading to the synthesis of functional proteins in models carrying nonsense mutations in cystic fibrosis transmembrane regulator (CFTR) (cystic fibrosis [CF]).

5.1.1 Pre-Clinical Data

In a large number of pharmacology studies, the translational read-through capabilities of ELX-02 have been tested in *in vitro* cell models and in *in vivo* animal models. ELX-02 induced expression of functional proteins with pharmacodynamic and behavioral effects with a satisfactory window of safety in several cellular and animal models of genetic disease caused by nonsense mutations, including, CF, cystinosis, Duchenne muscular dystrophy (DMD), mucopolysaccharidosis type I (MPS I), and Rett syndrome.

[redacted]

Further preclinical data on ELX-02 are available in the Investigator's Brochure (IB).[1]

5.1.2 Clinical Data

Eloxx Pharmaceuticals has completed two Phase 1a, first-in-human, randomized, double-blinded placebo-controlled single dose-escalating studies in healthy human subjects. These studies (combined) evaluated single doses of ELX-02 between 0.3 mg/kg and 7.5 mg/kg in 60 healthy subjects and characterized general and specialized safety parameters, and PK. In these studies, ELX-02 was generally well tolerated, showed typical PK parameters for an aminoglycoside, and showed an acceptable safety profile without severe or serious drug-related AEs. A Phase 1b, randomized, double-blinded placebo-controlled multiple dose-escalating study in healthy human subjects is ongoing.

In total, 25/40 (62.5%) subjects experienced at least 1 TEAE after administration of ELX-02 and 9/20 (45.0%) subjects after administration of placebo. The most frequently observed TEAEs were: injection site reaction, observed in 4/40 (10%) subjects after administration of ELX-02 and 2/20 (10%) subjects after administration of placebo, and headache, observed in 4/40 (10%) subjects after administration of ELX-02 and 1/20 (5%) subjects after administration of placebo.

Ototoxicity is a known complication associated with aminoglycoside use. Auditory function and vestibular function were assessed in the Phase 1a studies using a battery of tests. An abnormal audiogram was reported in 1 out of 7 subjects, 7 days after administration of ELX-02 0.3 mg/kg IV. The AE was considered mild and not related to study drug by the Investigator and resolved on Day 106 after the administration of study drug. One out of 8 subjects, at a dose level of 5 mg/kg showed a high frequency threshold shift on audiometry, and it remained unresolved on post-dose Day 58. There was no clinical impact, as there were no symptoms, and pure tone audiometry (normal speech frequencies) remained normal. This event was considered probably related to the study drug by the Investigator, the Sponsor did not consider it related to the study drug based on the opinion of the audiometry expert. This event was considered to be an SAE by the Investigator. There were no abnormal auditory findings noted at a dose level of 7.5 mg/kg. In addition to these events, ear discomfort was reported in 3 subjects and ear pain was reported in 3 subjects (7.1%, each): 5 of the 6 subjects with ear symptoms received ELX-02 while 1 subject received placebo.

The Phase 1a studies assessed the impact of ELX-02 dosing on clusterin and KIM-1, biomarkers of early proximal renal tubular injury. Although nephrotoxicity is a known complication of aminoglycoside use, there was no evidence in the completed single-dose studies that injection of ELX-02 affects renal function.

ELX-02 demonstrated linear dose PK in plasma with a short plasma half-life and parameters characteristic of an aminoglycoside. After single SC administrations, ELX-02 was rapidly absorbed with a median T_{max} of 0.5 hours for the lowest dose of 0.3 mg/kg and of 1 hour for the other doses. The elimination was rapid with mean terminal half-life ($T_{1/2}$) ranging between 2 to 4 hours for lower doses (0.3 to 5.0 mg/kg) whereas mean $T_{1/2}$ was longer for the highest dose (about 8 hours for 7.5 mg/kg) as expected with the use of a non-compartmental model in a situation of multiple exponential elimination. The decline of plasma concentrations was monophasic at lowest doses whereas it was biphasic for doses of 2.5 and 5.0 mg/kg and multiphasic for dose of 7.5 mg/kg with concentrations longer quantifiable at higher doses. Mean residence time was about 3 to 4 hours and mean apparent body clearance about 6 L/h for all doses. Mean apparent volume of distribution was dose-dependent with values of about

16.9-70.5 L for doses of 0.3 to 7.5 mg/kg. These data suggest a larger distribution with higher doses, not only restricted to extracellular fluids. The inter-subject variability on main plasma PK parameters (C_{max}, $AUC_{0\text{-}inf}$, partial AUCs) was low with CV% ranging from 6.54 to 18.6% for C_{max} and 7.64 to 18.2% for AUCs.

Renal excretion accounted for a large part of the eliminated drug. Mean percent of ELX-02 recovered in urine over the 48 hours post-dose of collection was 85.2% for IV treatment and ranged from 81.1 to 99.2% for SC doses. For all SC doses, more than 78% of the administered drug was excreted within the 12 hours post-dosing. Mean renal clearance was about 4.8 L/h for IV treatment and ranged between 4.6 to 6.1 L/h for SC treatments.

Further clinical data on ELX-02 from single dose studies are available in the IB.[1]

Presently, there is one ongoing clinical study with ELX-02 (study EL-002). It is a Phase 1, randomized, double-blinded, placebo-controlled, third party open, multiple-dose escalation, single center study to evaluate the safety, tolerability, and PK of subcutaneously administered ELX-02 in independent consecutive cohorts of healthy subjects.

The study includes at least 5 cohorts of 9 subjects each (both males and females need to be enrolled in each cohort, a significant number of female subjects need to be enrolled). Subjects will be randomized to receive multiple doses of ELX-02 or placebo at a ratio of 2:1 in each cohort. Six subjects in each cohort will receive ELX-02 and three will receive placebo. The cohorts are as follows:

Cohort 1 – ELX-02, 0.1 mg/kg or placebo SC twice a week for a total of nine doses;
Cohort 2 - ELX-02, 0.3 mg/kg or placebo SC twice a week for a total of nine doses;
Cohort 3 - ELX-02, 1.0 mg/kg or placebo SC twice a week for a total of nine doses;
Cohort 4 – ELX-02, 2.5 mg/kg (100 mg/mL) or placebo SC twice a week for a total of nine doses;
Cohort 5 - ELX-02, up to 2.5 mg/kg (50 mg/mL per injection) or placebo SC twice a week for a total of nine doses;
Cohort 6 - ELX-02, 2.5 to 5.0 mg/kg ELX-02 or placebo SC twice a week for a total of nine doses;
Cohort 7 - ELX-02, up to 5.0 mg/kg or placebo SC twice a week for a total of nine doses.

Dose escalation to the next cohort will not proceed until after the 14-day observation period for the previous cohort in order to allow for the detection of unanticipated delayed AEs. Changes to the planned dose may be made in any cohort, depending on available safety and PK data. The dose will not exceed 5.0 mg/kg. Additional cohorts of subjects may be considered to further establish the safety of ELX-02.

As of the date of this protocol, the first 4 dosing cohorts have been completed. The study remains blinded (except for one subject with AE of abnormal audiometry that was unblinded by the Sponsor) and the available data are preliminary. [redacted]

- [redacted]
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[redacted]

5.1.3.2 Benefits

ELX-02 is being developed for patients with genetic disorders caused by nonsense mutations, and this study will help in the development of the drug. There are no direct benefits for the subjects participating in this study.

5.2 Study Rationales

5.2.1 Rationale for Study Design

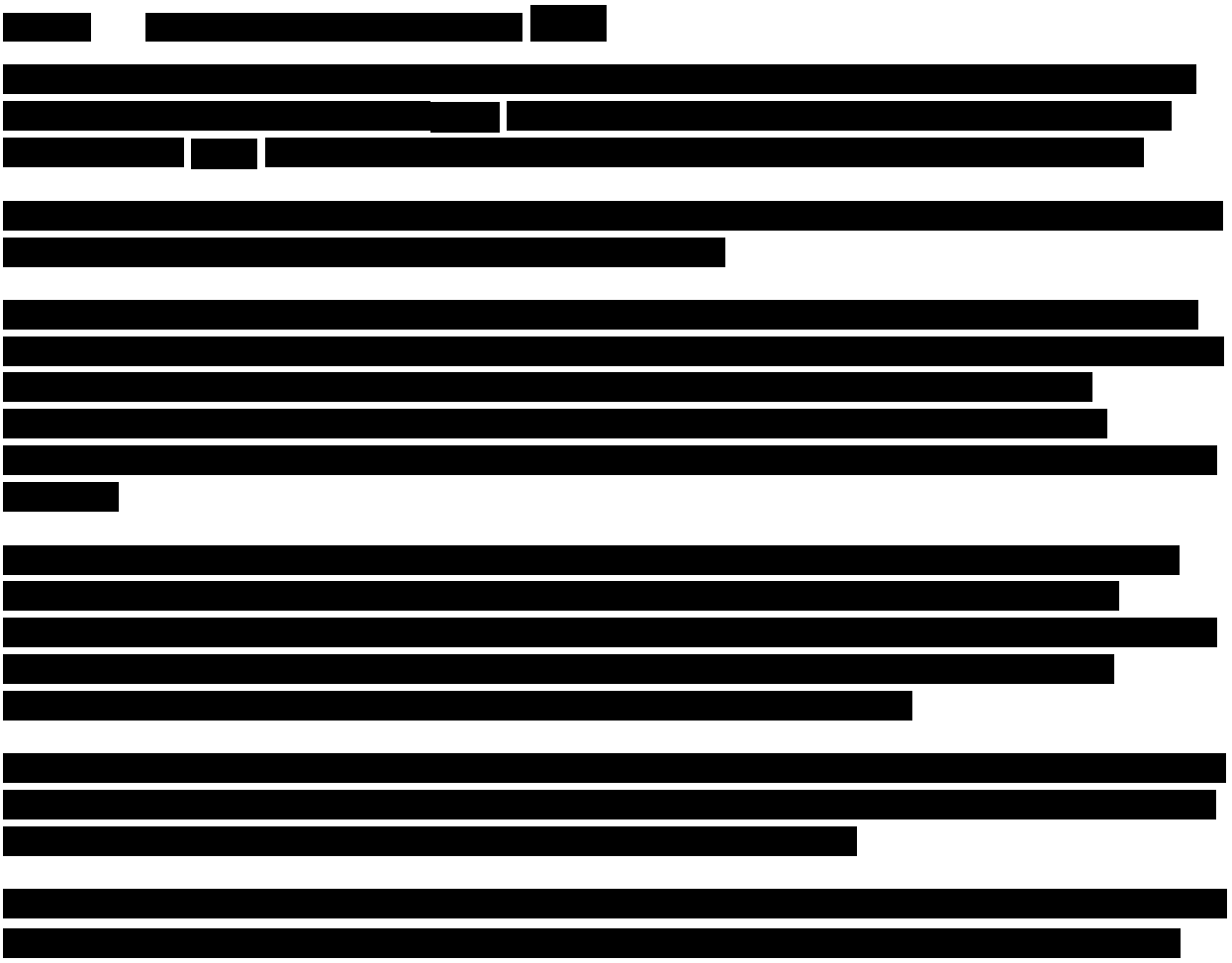
Aminoglycosides are known to produce nephrotoxicity in experimental animals and man at high doses and after repeated doses, and the risk of nephrotoxicity is generally greater in patients with impaired renal function and in those who receive high dosage of prolonged therapy.[1,2] ELX-02 is a synthetic aminoglycoside that is eliminated primarily through renal excretory mechanisms.[1]

The clinical development program of ELX-02 includes Phase 2, proof-of-principle, dose finding studies in patients with CF and cystinosis.[1] Before starting the first Phase 2 study involving the administration of ELX-02 doses from 1 mg/kg up to 5 mg/kg over 4 weeks in patients with mild to moderate nephropathic cystinosis with at least 1 nonsense mutation in the cystinosin gene, the influence of renal impairment in relation to the PK of the drug will be evaluated. This is because when there is a risk of clinically relevant increases in exposure to a drug or active metabolite in patients with renal impairment, the FDA Draft *Guidance for Industry, Pharmacokinetics in Patients with Impaired Renal Function - Study Design, Data Analysis, and Impact on Dosing and Labeling*[3] and the EMA *Guideline on the evaluation of the pharmacokinetics of medicinal products in patients with decreased renal function*[4] recommend the conduct of a PK study in subjects with decreased renal function in order to obtain adequate treatment recommendations.

This Phase 1 study is designed to determine the effect of various severities of renal impairment (i.e., mild, moderate, and severe renal impairment, as compared to a control group with normal renal function) on the PK of ELX-02 after a single dose of 1 mg/kg. The study will also gather additional data on the safety and tolerability of ELX-02 in subjects with normal renal function, and preliminary safety and tolerability in RI subjects, by evaluating AEs, local reactions at the injection site, physical examination, vital signs, ECG, and clinical laboratory parameters. This study should allow the development of specific dosing recommendations across the entire spectrum of renal impairment.

5.2.2 Rationale for Dose Selection

A single-dose study to evaluate the effects of renal impairment is appropriate if the drug and its active metabolites (if any) are expected to exhibit dose-linear and time-independent PK in RI individuals, and steady state PK can be predicted from single-dose data.[4] ELX-02 showed dose-exposure linearity in the range of 0.3 mg/kg to 7.5 mg/kg following single-dose administration. Based on pre-clinical data, the anticipated clinically efficacious doses range from 2.5 mg/kg to 5.0 mg/kg, which are within the dose proportionality range. At pharmacological doses, ELX-02 shows no accumulation in plasma and a relatively low accumulation in the kidney and cochlear tissues.[1] Based on these properties, a single-dose design is judged appropriate to assess the influence of renal impairment on the PK of ELX-02.



[redacted]

5.2.2.2 Anticipated Exposure

ELX-02 is primarily eliminated via the kidney similar to what has been observed for other aminoglycosides. The main processes are glomerular filtration and subsequent reabsorption in the kidney. Aminoglycosides are reabsorbed in the proximal tubules in the kidney via megalin, a transmembrane endocytic receptor expressed in epithelial membrane in the kidney as well as other tissues. In addition to megalin, other transporters expressed in the kidney (OCT2 and SGLT2) play a role in the reabsorption of aminoglycosides in the kidney.

A dose of 1 mg/kg of ELX-02 has been selected for the present study as it has been shown to allow for a good characterization of ELX-02 PK profile. In the Phase 1 SAD study noted above, following a single 1 mg/kg SC dose, the mean (N=6) C_{max} was 3576 (± 502) ng/mL, the median T_{max} was 1.0 (range 1.0-1.0) hours, and the mean AUC_{0-12} was 13,131 (± 1729) ng*h/mL. The half-life was 2.14 (± 2.14) hours, and clearance (CL/F) was 5.50 (± 0.349) L/h.[1]

[redacted]

5.2.3 Safety Measures to be Utilized during the Present Study

[redacted]The mild (Group 1) and moderate (Group 2) patients with renal disease will be dosed first, in a parallel fashion. At this point, a safety committee composed of Sponsor and CRO members will jointly review the PK data before dosing the patients with severe renal disease (Group 3). For the control group (Group 4) a mean matching procedure will be performed in order to match the healthy subjects in the control group with the RI subjects.

5.2.4 Rationale for the Study Population

This study will be conducted in patients with mild, moderate, or severe renal impairment, as well as in age and BMI matched healthy controls. Most drugs are cleared by elimination of unchanged drug by the kidney and/or metabolism in the liver and/or small intestine. In patients with impaired renal function, the PK of a drug that is eliminated primarily by the kidney may be altered to an extent that the dosing regimen may need to be changed. The primary change

resulting from renal impairment is a decrease in renal excretion of the drug or its metabolites, but changes in renal excretion of a drug may also occur. Renal impairment may also be associated with changes in absorption, plasma protein binding, transport and tissue distribution. Therefore, the FDA recommends that PK be assessed in patients with renal impairment in order to provide proper dosing recommendations.

6. Objectives

Primary objective:

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

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.

7. Study 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 subjects enrolled in the study will be categorized in 4 groups according to their renal function (eGFR value calculated using the MDRD4 equation – refer to section 9.2):

Group 1: subjects with mild renal impairment
Group 2: subjects with moderate renal impairment
Group 3: subjects with severe renal impairment
Group 4 (control group): subjects with normal renal function

The mild (Group 1) and moderate (Group 2) patients with renal disease will be dosed first, in a parallel fashion. At this point, interim PK analyses will be performed and a safety committee composed of Sponsor and CRO members will jointly review the PK data. [redacted]
The safety committee will then authorize dosing in patients with severe renal disease (Group 3). Control subjects (Group 4) will be recruited after the recruitment of Groups 1 to 3 to facilitate subjects' matching between the healthy subjects in the control group with the RI subjects.

Each subject will receive a single SC dose of ELX-02 1 mg/kg on Day 1. They will remain as in-patients at the clinical site under close surveillance by the site staff for 72 hours post-dose and they will come back for a follow-up visit on Day 8 (±1 day).

Serial blood and urine samples will be collected to quantify ELX-02, in order to evaluate the effect of the various severities of renal impairment (i.e., mild, moderate, and severe renal impairment, as compared to a control group with normal renal function) on the PK of study drug. The study will also evaluate the safety and tolerability of ELX-02 in subjects with normal renal function and in RI subjects, by evaluating AEs, local reactions at the injection site, physical examination, vital signs, ECG, early markers of renal injury, and clinical laboratory parameters.

This study is intended for filing under FDA regulations.

8. Study Population

8.1 Sample Size

It is targeted to enroll approximately 24 to 26 healthy adult male or female volunteers.

Subjects will be 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 who withdraw or are withdrawn from the study after dosing, for reasons other than safety and tolerability, may be replaced in order to ensure a minimum of 6 completed subjects per group. The total number of subjects dosed (including potential replacement subjects) will remain within a maximum of 8 subjects per group and within a maximum of 32 subjects for the whole study.

8.2 Subjects with Renal Impairment (Groups 1 to 3)

Subjects enrolled in Groups 1, 2, and 3 will be members of the community at large. The recruitment advertisements may use various media types (e.g. radio, newspaper, Web site or volunteer database of the University of Miami).

8.2.1 Inclusion Criteria for Subjects with Renal Impairment (Groups 1 to 3)

Subjects must meet all of the following criteria to be included in the study Groups 1 to 3:

1) Male or female, non-smoker and/or light smoker (up to 5 cigarettes or equivalent/day), ≥ 18 and ≤ 80 years of age, with BMI ≥ 18.0 and ≤ 40.0 kg/m^2 and body weight ≥ 50.0 kg for males and ≥ 45.0 kg for females.
2) Have a diagnosis of renal impairment that has been stable, without any significant change in overall disease status in the last 3 months prior to screening as determined by the PI.
3) Have an eGFR expressed in mL/min/1.73 m^2 (MDRD4 equation) at screening within the range of:
   a) Group 1 - Mild Group: 60 - 89 mL/min/1.73 m^2;
   b) Group 2 - Moderate Group: 30 - 59 mL/min/1.73 m^2;
   c) Group 3 - Severe Group: < 30 mL/min/1.73 m^2 not requiring dialysis.

   eGFR results that are deemed inconsistent with the usual stage of renal impairment may be repeated. Subjects are categorized into severity group at screening. If the eGFR scores change on Day-1 or other visit due to a non-clinically significant change in clinical status or laboratory result, the subject keeps the original severity group.
4) Subject may have stable treated medical illnesses and underlying diseases producing the renal impairment such as diabetes, hypertension, or cardiovascular disease, providing that, in the opinion of the PI, the disease is stable.
5) Have normal or non-clinically significant findings at physical examination, vital signs and ECG and normal limits or non-clinically significant deviations in clinical laboratory

evaluations at screening, with the exception of findings that in the opinion of the PI are consistent with subject's renal impairment or due to other stable diseases. Clinical data, ECG and laboratory tests may be repeated at the discretion of the Investigator.

6) Other than renal impairment, have no other conditions which may significantly impact study drug absorption or metabolism, as determined by the PI.

7) Stable medical regimen, deemed not to interact with study drug PK, for 14 days prior to dosing, except for routine daily management of electrolytes (e.g. potassium), acid-base, or other associated disorders expected in patients with renal impairment. The approval of the medications for the management of renal impairment and the treatment of concomitant stable medical conditions (e.g. diabetes, hypertension, associated stable cardiovascular disease, stable psychiatric conditions) is at the discretion of the PI.

8) Females of childbearing potential who are sexually active with a non-sterile male partner (sterile male partners are defined as men vasectomized since at least 6 months) must be willing to use one of the following acceptable contraceptive method throughout the study and for 30 days after study drug administration:

   a) simultaneous use of intra-uterine contraceptive device, without hormone release system, placed at least 4 weeks prior to study drug administration, and condom for the male partner;

   b) simultaneous use of diaphragm with intravaginally applied spermicide and male condom for the male partner, starting at least 21 days prior to study drug administration.

9) Male subjects who are not vasectomized for at least 6 months, and who are sexually active with a non-sterile female partner (sterile female partners include post-menopausal females and surgically sterile females) must be willing to use one of the following acceptable contraceptive method from dosing until at least 90 days after study drug administration:

   a) simultaneous use of a male condom and, for the female partner, hormonal contraceptives (used since at least 4 weeks) or intra-uterine contraceptive device (placed since at least 4 weeks);

   b) simultaneous use of a male condom and, for the female partner, a diaphragm with intravaginally applied spermicide.

10) Male subjects (including men who have had vasectomy) with a pregnant partner must agree to use a condom from dosing until at least 90 days after study drug administration.

11) Male subjects must be willing not to donate sperm until 90 days following study drug administration.

12) Able to understand and willing to sign the ICF and comply with the study restrictions.

8.2.2 Exclusion Criteria for Subjects Renal Impairment (Groups 1 to 3)

Subjects to whom any of the following applies will be excluded from the study:

1) Unstable renal function or acute exacerbation of renal disease within 14 days of study drug administration, as indicated by recent history or worsening of clinical and/or laboratory signs of renal impairment as judged by the PI. For inclusion and for categorization by MDRD4 or

other equations, laboratory results that are deemed inconsistent with the usual stage of renal impairment may be repeated.

2) Has a functioning renal transplant.
3) Major illness or surgery within 4 weeks prior to dosing.
4) Clinically significant unstable medical condition or history of any illness that may increase the risk associated with study participation or investigational drug administration or may interfere with the interpretation of study results and, in the judgment of the PI, would make the subject inappropriate for entry into this study. Subjects who do not qualify based on a reversible medical condition or mild inter-current illness may be re-evaluated after further testing/examination or re-screened after the condition is resolved.
5) Positive test for hepatitis B, hepatitis C, or HIV at screening.
6) History of allergic reactions, hypersensitivity or toxic reactions to aminoglycosides.
7) History of anaphylaxis.
8) Supine 12-lead ECG abnormalities at screening considered clinically significant by the PI.
9) Clinically significant vital sign abnormalities (systolic blood pressure lower than 90 or over 160 mmHg, diastolic blood pressure lower than 40 or over 100 mmHg, or heart rate less than 45 or over 100 bpm) at screening.
10) History of significant drug or alcohol abuse within six months prior to screening.
11) Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days (or 5 half-lives, whichever is longer) prior to dosing, administration of a biological product in the context of a clinical research study within 90 days prior to dosing, or concomitant participation in an investigational study involving no drug or device administration.
12) Positive urine drug screen or alcohol test at screening, unless the positive drug screen is due to prescription drug use that is documented and approved by the PI.
13) Female subject with positive pregnancy test at screening.
14) Breast-feeding or pregnant subject within 6 months prior to study drug administration.
15) Use of any drugs known as strong inducer or inhibitor of hepatic drug metabolism within 30 days prior to study drug administration.
16) Use of medication other than stable medications approved by the PI and topical products without significant systemic absorption:
    a) any new prescription medication within 14 days prior to dosing;
    b) OTC products or natural health products (including herbal remedies such as St. John's wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) within 7 days prior to dosing, with the exception of the occasional use of acetaminophen (that is allowed up to 2 g daily), unless the products are used by RI subjects as part of their routine care;
    c) a depot injection or an implant of any drug within 3 months prior to dosing.

17) The following medications are prohibited during the study: neuromuscular blocking agents (e.g. succinylcholine, tubocurarine), neurotoxic and/or nephrotoxic drugs (e.g. other aminoglycosides, cisplatin, cephaloridine, polymyxin B, vancomycin, viomycin). At the discretion of the PI, a suitable substitute medication may be prescribed to replace the prohibited medication.

18) Donation of plasma within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to the first dosing.

19) Any reason which, in the opinion of the PI, would prevent the subject from participating in the study.

20) Inability to be venipunctured and/or tolerate catheter venous access.

21) Presence of mitochondrial mutation(s) making the subject susceptible to aminoglycoside toxicity.

22) Presence of signs of dehydration, recent history of neuromuscular blockade or clinically significant history of vestibular impairment.

8.3 Subjects with Normal Renal Function (Group 4)

An effort will be made to carefully match the healthy subjects in the control group with the RI subjects by age (± 10 years), BMI (±15%), and gender, to the extent possible. A mean matching procedure will be performed. Subjects enrolled in this group of study will be members of the community at large. The recruitment advertisements may use various media types (e.g. radio, newspaper, inVentiv Web site, inVentiv volunteer database).

8.3.1 Inclusion Criteria for Subjects with Normal Renal Function (Group 4)

Subjects must meet all of the following criteria to be included in the study Group 4:

1) Male or female, non-smoker (no use of tobacco or nicotine products within 3 months prior to screening), ≥ 18 and ≤ 80 years of age, with BMI ≥ 18.0 and ≤ 40.0 kg/m^2 and body weight ≥ 50.0 kg for males and ≥ 45.0 kg for females.
2) Have an eGFR ≥ 90 mL/min/1.73 m^2 (MDRD4 equation).
3) Healthy as defined by:
   a) the absence of clinically significant illness and surgery within 4 weeks prior to dosing. Subjects vomiting within 24 hours pre-dose will be carefully evaluated for upcoming illness/disease. Inclusion pre-dosing is at the discretion of the PI.
   b) the absence of clinically significant history of hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, and immunologic disease.
4) Matched to subjects with RI (mild, moderate or severe) according to gender, age (± 10 years), and BMI (± 15%).
5) Females of childbearing potential who are sexually active with a non-sterile male partner (sterile male partners are defined as men vasectomized since at least 6 months) must be

willing to use one of the following acceptable contraceptive method throughout the study and for 30 days after study drug administration:

a) simultaneous use of intra-uterine contraceptive device, without hormone release system, placed at least 4 weeks prior to study drug administration, and condom for the male partner;

b) simultaneous use of diaphragm with intravaginally applied spermicide and male condom for the male partner, starting at least 21 days prior to study drug administration.

6) Male subjects who are not vasectomized for at least 6 months, and who are sexually active with a non-sterile female partner (sterile female partners include post-menopausal females and surgically sterile females) must be willing to use one of the following acceptable contraceptive method from dosing until at least 90 days after study drug administration:

a) simultaneous use of a male condom and, for the female partner, hormonal contraceptives (used since at least 4 weeks) or intra-uterine contraceptive device (placed since at least 4 weeks);

b) simultaneous use of a male condom and, for the female partner, a diaphragm with intravaginally applied spermicide.

7) Male subjects (including men who have had vasectomy) with a pregnant partner must agree to use a condom from the first dosing until at least 90 days after study drug administration.

8) Male subjects must be willing not to donate sperm until 90 days following study drug administration.

9) Able to understand and willing to sign the ICF and comply with the study restrictions.

8.3.2 Exclusion Criteria for Subjects with Normal Renal Function (Group 4)

Subjects to whom any of the following applies will be excluded from the study:

1) Any clinically significant abnormality at physical examination or clinically significant abnormal laboratory test results at screening.

2) Positive test for hepatitis B, hepatitis C, or HIV at screening.

3) History of allergic reactions, hypersensitivity or toxic reactions to aminoglycosides.

4) Evidence or history of clinically relevant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, immunologic, or allergic disease. This includes any acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational drug administration or may interfere with the interpretation of study results and, in the judgment of the PI, would make the subject inappropriate for entry into this study.

5) History of anaphylaxis.

6) Clinically significant supine 12-lead ECG abnormalities at screening, e.g., QTcF >450 msec for men and >470 msec for women, or a QRS interval >120 msec.

7) Clinically significant vital sign abnormalities (systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 50 or over 90 mmHg, or heart rate less than 50 or over 100 bpm) at screening.
8) History of significant alcohol abuse within one year prior to screening or regular use of alcohol within six months prior to the screening visit (more than fourteen units of alcohol per week [1 unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol]).
9) History of significant drug abuse within one year prior to screening or use of soft drugs (such as marijuana) within 3 months prior to the screening visit or hard drugs (such as cocaine, PCP, crack, opioid derivatives including heroin, and amphetamine derivatives) within 1 year prior to screening.
10) Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days (or 5 half-lives, whichever is longer) prior to dosing, administration of a biological product in the context of a clinical research study within 90 days prior to dosing, or concomitant participation in an investigational study involving no drug or device administration.
11) Positive urine drug screen, alcohol test, or urine cotinine test at screening.
12) Female subject with positive pregnancy test at screening.
13) Breast-feeding or pregnant subject within 6 months prior to study drug administration.
14) Use of any drugs known to induce or inhibit hepatic drug metabolism within 30 days prior to study drug administration.
15) Use of medication other than topical products without significant systemic absorption:
    a) prescription medication within 14 days prior to dosing;
    b) OTC products and natural health products (including herbal remedies such as St. John's wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) within 7 days prior to dosing, with the exception of the occasional use of acetaminophen (that is allowed up to 2 g daily);
    c) a depot injection or an implant of any drug within 3 months prior to dosing.
16) Donation of plasma within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to dosing.
17) Hemoglobin < 12.8 g/dL (males) and < 11.5 g/dL (females) and hematocrit < 37% (males) and < 32% (females) at screening.
18) Any reason which, in the opinion of the PI, would prevent the subject from participating in the study.
19) Inability to be venipunctured and/or tolerate catheter venous access.
20) Presence of mitochondrial mutation(s) making the subject susceptible to aminoglycoside toxicity.

21) Presence of signs of dehydration, recent history of neuromuscular blockade or clinically significant history of vestibular impairment.

9. Clinical Procedures

The study will be conducted jointly at inVentiv and the Division of Clinical Pharmacology of the University of Miami. Groups 1 to 3 study procedures will be conducted at the Division of Clinical Pharmacology of the University of Miami (identified as Site 01) and Group 4 study procedures will be conducted at inVentiv (identified as Site 02). Clinical procedures, data collection and evaluation will be performed as per applicable SOPs of the Division of Clinical Pharmacology of the University of Miami and inVentiv, as appropriate, unless specified otherwise.

9.1 Screening Procedures

Subject screening procedures will be performed within 35 days preceding administration of study medication. Subjects must provide written informed consent prior to initiation of any screening procedures. The consent to perform some general screening procedures may be obtained on a consent document other than the ICF specific to this study, and therefore, some screening test results could be obtained before signature of the ICF specific to this study. The study-specific ICF must be signed and dated by the subject before participation to study-specific procedures.

Screening procedures will include: demographic data, medical and medication histories, complete physical examination, body measurements, ECG (12-lead), vital signs (BP, HR, RR, OT), hematology, biochemistry, coagulation, HIV, hepatitis B and C tests, urinalysis, genetic testing for mitochondrial mutations, urine drug screen, alcohol test (breath or urine), urine pregnancy test, and urine cotinine test (for Group 4 only).

Serum cystatin C will be measured at screening and cystatin GFR will be estimated for additional data analysis, only if needed. These values will have no impact on subject's eligibility and categorization.

For eligibility purposes, abnormal laboratory or vital signs results may be repeated once (subjects of Groups 1 to 3 may need more repeats to confirm stability) if abnormal result is observed at the initial reading. Moreover, abnormalities found in the ECG may need to be confirmed by repeated measurements. In the event that the participation of a subject in the study is delayed and some screening procedures had been performed outside the prescribed screening window, outdated screening procedures can be repeated.

Subjects from Groups 1 to 3 (mild, moderate, and severe RI subjects) should be recruited prior to recruiting control group subjects in order to facilitate subjects' matching.

Subjects of Groups 1 to 3 who do not qualify based on a reversible condition or mild inter-current illness may be re-evaluated after further testing/examination or re-screened after the condition is resolved.

9.2 Assessment of Renal Function for Eligibility and Categorization

Renal function will be assessed at screening. Subjects will be classified in one of the 4 study groups according to their eGFR obtained from the MDRD4 study equation (Table 2) based on

serum creatinine. 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 2. Classification of Subjects in Groups According to Renal Function

Group	Description	eGFR ($mL/min/1.73m^2$)	Number of Subjects
1	Mild decrease in GFR	60-89	6
2	Moderate decrease in GFR	30-59	6
3	Severe decrease in GFR, not requiring dialysis	< 30; not requiring dialysis	6
4	Control (normal) GFR	≥ 90	6-8

MDRD4 (4-variable) equation:

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

GFR is expressed as $mL/min/1.73\ m^2$ of BSA
Scr is serum creatinine expressed in mg/dL
Age is expressed in years.

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

9.3 In-Patient Stay and Visit

Subjects will remain as in-patients from the morning of Day -1 until after the 72-hour post-dose blood draw. Subjects will come back for a follow-up visit on Day 8 (±1 day).

Participation of each subject in this study should last approximately 1 week (approximately 5 weeks including the screening visit).

9.3.1 Outings

For subjects in Groups 1 to 3: Short outings will be permitted during the in-patient period for smoking subjects. Non-smokers could also go out for supervised outings at the discretion of the site staff. Outings will be supervised at all times by the clinical staff to ensure compliance with protocol and will be limited to the grounds surrounding the site, as per the clinical site specific procedures for supervised outings.

9.4 Randomization, Blinding, and Matching

This study will be open-label due to the objective nature of the data. Subjects will be enrolled into the trial and assigned to one of 4 groups in a non-randomized fashion.

Subjects will be assigned to one of the 4 study groups (Group 1, 2, 3, or 4) according to their eGFR value, as per Table 2.

Subjects with normal renal function (Group 4) will be matched with RI subjects by age (± 10 years), gender, and BMI (± 15%). A mean matching procedure will be performed. Approximately 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).

9.5 Study Medication

Treatment: ELX-02 ready-to-use, sterile, solution for injection [redacted]

Dose: Single SC dose of 1 mg/kg ELX-02

9.6 Drug Supplies and Accountability

It is the responsibility of the Sponsor to ensure that study medication provided for this study are manufactured under Good Manufacturing Practices (GMP) and are suitable for human use. It is the responsibility of the Sponsor to ship a sufficient amount of dosage units to allow the clinical sites to conduct the study. Study medication will be stored by the clinical sites as per the labeled storage conditions and the pharmacy manual.

The medications will be stored in a locked, environmentally-controlled medication room with restricted access. Container(s) will bear a label containing at least the name of the study drug, lot and/or batch number, and expiry/retest date. One single-use vial per subject will be identified with at least the project number and the subject number/spare number. The medications will be dispensed according to the clinical site SOP and prepared according to the pharmacy manual.

All study drug received at the site will be inventoried and accounted for throughout the study and the result recorded in the drug accountability/retention record according to the clinical site appropriate SOP. Upon completion of the study, the remaining drug products will be maintained at the clinical sites, destroyed or returned to the Sponsor, as per Sponsor's request.

9.7 Drug Administration

Subjects will receive a single dose of 1 mg/kg ELX-02 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.

[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.

9.8 Study Restrictions

9.8.1 Food and Fluids

For standardization purpose, ELX-02 will be administered at least 30 minutes after subjects have been served a light breakfast. No food will be allowed until at least 1 hour after dosing. Standardized meals will be served at appropriate times thereafter.

To keep subjects well hydrated, they will be asked to consume 240 mL of fluids within 120 minutes prior to ELX-02 dosing (including fluids served as part of the breakfast). The fluids will be water or a fruit beverage should the PI or designee judges that such beverage is more appropriate to prevent dizziness and fainting. Water will be provided *ad libitum* at all other times, however the study staff will encourage subjects to drink at least 200 mL of fluids at the beginning of each 12-hour post-dose period.

In addition, subjects will be required to abstain from:

- food containing poppy seeds within 24 hours prior to admission;
- food or beverages containing xanthine derivatives or xanthine-related compounds (coffee, black/green tea, chocolate) or energy drinks from 48 hours prior to dosing until after the 72-hour blood sample collection;
- natural health products (including herbal remedies such as St. John's wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) from 7 days prior to dosing until study exit;

- food or beverages containing grapefruit, Seville orange, starfruit, pomegranate, pineapple, or pomelo from 7 days prior to dosing until after the 72-hour blood sample collection.

9.8.2 Tobacco, Alcohol, and Illicit Drugs

For Groups 1 to 3, subjects will be required to abstain from using soft or hard drugs from screening and throughout the study. For light smoking subjects, smoking will be prohibited from at least 2 hours pre-dose until 2 hours post-dose and a maximum of 5 cigarettes or equivalent per day will be allowed while subjects are in-patients in the clinic.

For Group 4, subjects will be required to abstain from using soft or hard drugs and from smoking from screening and throughout the study.

Consumption of alcohol-based products will be prohibited from 24 hours prior to admission until after the 72-hour blood sample collection.

9.8.3 Concomitant Medications

Stable concomitant medical treatment is allowed for subjects of Groups 1 to 3, provided that a) no changes in their dosing regimen occurred for at least 14 days prior to dosing, and no changes are expected throughout the study, other than for routine daily management of electrolytes (e.g. potassium), acid-base, or other associated disorders expected in patients with renal impairment; b) the medications are deemed not to interact with study drug PK; and c) the medications have been approved by the PI before the subject inclusion. The following medications are prohibited during the study: neuromuscular blocking agents (e.g. succinylcholine, tubocurarine), neurotoxic and/or nephrotoxic drugs (e.g. other aminoglycosides, cisplatin, cephaloridine, polymyxin B, vancomycin, viomycin). If a subject is taking an excluded medication, the PI can switch the subject to an alternate medication that is allowed and the subject can be screened for the study after appropriate stabilization on the new medication. This will be done after the subject signs the ICF.

No other concomitant drug therapy (new prescription or OTC medications) will be allowed for subjects of Groups 1 to 3, except one(s) required for the medical management of an AE or a new medical condition.

For subjects of Group 4, prescription and OTC medications will be prohibited throughout the study. No concomitant drug therapy will be allowed during the study except one(s) required for the medical management of an AE.

Any concomitant medication use (in Groups 1 to 4) other than the occasional use of acetaminophen will be evaluated on a case-by-case basis by the PI or a Sub-Investigator. All concomitant medication use will be documented from screening through study exit / early termination.

9.8.4 Posture and Physical Activity

Subjects will be allowed to engage in normal activity but will avoid lying down or sleeping, unless medically necessary or procedurally required, for 4 hours after drug administration. Vigorous activity will be prohibited at all times during the in-house portion of the study. Because

excessive physical activity may increase the level of CPK above the upper normal limit value, subjects will be advised to avoid performing such activity (e.g., high-intensity running, biking, weightlifting) at all times during the study duration.

9.8.5 Contraception

In the reproduction phase of toxicology studies conducted in juvenile rats, there were no adverse ELX-02-related effects noted on sexual maturation, reproductive and fertility indices, maternal uterine examinations, or sperm evaluations.[1] However, since there are no adequate and well-controlled studies with pregnant women, it is uncertain whether there is human fetal risk associated with the use of ELX-02. However, aminoglycosides are known to be associated with fetal deformities, therefore, non-pregnant, non-lactating females will be included in the study. Female subject of childbearing potential will be included only if they use appropriate methods of contraception.

Male subjects who are not vasectomized will also be required to use appropriate methods of contraception during the study to avoid pregnancies in their female partners. Male subjects (including men who have had vasectomy) with a pregnant partner must agree to use a condom to prevent *in utero* drug exposure.

9.9 Sample Collection for Pharmacokinetic Evaluation

9.9.1 Blood Sample Collection and Processing

A total of 13 blood samples will be drawn from each subject of Groups 1 to 4 for PK analyses. Blood samples will be collected 0.25, 0.5, 0.75, 1, 2, 4, 6, 12, 24, 36, 48, 72, and 168 (Day 8) hours post-dose (up to 6 mL for each sampling time).

The time tolerance window for blood samples will be ±1 minute for timepoints up to 2 hours post-dose; ±5 minutes from 4 hours to 12 hours; ± 10 minutes from 24 hours to 72 hours; and ± 24 hours for the 168-hour sample. Sample collections done outside the pre-defined time windows will not be considered as protocol deviations since actual post-dose sampling times will be used for PK and statistical analyses. Unless otherwise specified or for subject safety, when blood draws and other procedures coincide, blood draws will have precedence. A saline intravenous catheter may be used for blood collection to avoid multiple skin punctures, when appropriate. Otherwise, blood samples will be collected by direct venipuncture.

The total volume of blood including that collected for eligibility and safety purposes should not exceed 196 mL for the whole study (refer to Table 3 for details). Deviations related to the volume of blood collected will be reported only when the total volume collected from a subject for the whole study is exceeded.

Table 3. Blood Volume for Laboratory Analyses

Analysis	Approximate Amount of Blood per Sample (mL)	Number of Timepoints	Total Amount of Blood (mL)
Hematology	4	3	12
Biochemistry	8.5	4	34
Coagulation	2.7	3	8.1
Serology	8.5	1	8.5
Genetic testing for mitochondrial mutations	5.4	1	5.4
PK	6	13	78
Extra volume for potential repeat draws or additional safety tests	-	-	50
Total Volume			196

Samples will be collected and processed as per the instructions given in the laboratory manual.

9.9.2 Urine Sample Collection and Processing

Urine samples will be collected for quantitation of unchanged ELX-02 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 samples will be collected as follows:

- At each void, all urine will be collected into a collection cup. The weight of each sample will be recorded and the volume of urine calculated assuming specific gravity of 1.
- Subjects will be asked to void their bladder within 10 minutes before the end of each collection interval, so that each new interval begins with an empty bladder.
- When a urine aliquot is needed at a specific timepoint for renal injury biomarkers and creatinine (pre-dose and at 12, 24, 36, and 48 hours post-dose), the aliquot will be withdrawn from the urine collection cup. If a subject is unable to void at the specified timepoint, it will be duly recorded and renal injury biomarkers and creatinine will be analyzed in the subsequent urine sample. Residual urine will be used for pooling with other voids of the same collection interval.
- All voids collected during a defined time interval will be pooled into one urine collection container. The sum of individual samples' weights will be recorded. Any urine voided by subjects at the intersection (within 10 minutes) of two intervals will be included in the earlier sample. Two aliquots will be taken in each pooled time interval container for PK analysis.
- Thereafter, all urine collected in the intervals 0-3, 3-6, 6-9, 9-12, 12-18, 18-24 will be pooled together to withdraw a sample for the calculation of creatinine clearance. The sum of all samples' weights will be recorded. Refer to section 9.10.8.

- At approximately 24 and 72 hours post-dose, a mid-stream safety sample will also be collected for urinalysis.
- Any urine voided by subjects but not collected will be documented.

Urine samples will be collected and processed as per the instructions given in the laboratory manual.

9.10 Subject Monitoring

Subjects will be monitored throughout the study by the clinical sites staff for AEs. The PI or a Sub-Investigator will be on site/campus for drug administration and until 4 hours after administration of the study medication to the last subject. The PI or a Sub-Investigator will also be on call for the remainder of the study. If necessary, a physician, either at the clinical site or in a nearby hospital will administer treatment for any AE(s). A crash cart or emergency bag containing the necessary rescue material and appropriate medications will be available in the clinic to allow rapid intervention in case of emergency.

Safety parameters, including laboratory results and ECG, will be assessed by the PI or delegate, using the clinical site criteria for biomedical laboratory and ECG acceptance ranges as suggested guidelines in making the medical assessment.

Scheduled safety measurements will be repeated according to appropriate clinical site SOPs or upon request from a physician. Any abnormal repeated measurement will be evaluated by a physician and repeated if judged necessary. Further action may be taken upon physician's request.

Subjects will be advised to notify their health care professional(s) (e.g., physician, dentist, and/or pharmacist) that they are participating in a clinical research study on an experimental drug called ELX-02 being developed for the treatment of genetic diseases before taking any medicines or undergoing any medical procedure.

9.10.1 Vital Signs

Blood pressure, respiratory rate, heart rate, and oral temperature will be measured 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. When vital signs measurements coincide with a blood draw, they should preferably be performed before the blood collection whenever possible. A time window of 15-20 minutes for vital signs will be allowed if there are multiple procedures at the same time point.

9.10.2 ECG

12-lead ECG will be performed at screening, prior to dosing, approximately 2 hours post-dose, at check-out on Day 4 and at study exit. ECG will be performed after a resting time of at least 5 minutes in supine position. When ECG coincides with a blood draw, it should preferably be performed before the blood collection whenever possible. A time window of 15-20 minutes for ECG will be allowed if there are multiple procedures at the same time point.

9.10.3 Physical Examination and Body Measurements

A complete physical examination will be performed at screening. A complete physical examination includes assessments of at least the following: head, eyes, ears, nose, throat (HEENT), neck, chest, lungs, abdomen, musculoskeletal, dermatological, cardiovascular/peripheral vascular, and general neurological examination.

A brief physical examination will be done on Day -1, at check-out on Day 4 and at study exit. A brief physical examination includes assessments of the following: HEENT, chest, lungs, abdomen, dermatological, cardiovascular/peripheral vascular, and areas of note elicited from the subject.

Body weight and height will be measured at screening and the BMI calculated. Body weight measurement will be done again on Day -1 and this value will be used to calculate the exact individual dose of ELX-02 required on a mg/kg basis.

9.10.4 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 hours post-dose, and at study exit. The extent of local reaction at the injection site will be graded according to the Division of AIDS (DAIDS) criteria[5] presented in APPENDIX 1.

9.10.5 Drug, Alcohol and Cotinine Screen

A urine drug screen (amphetamines, methamphetamines, barbiturates, benzodiazepines, tetrahydrocannabinol, cocaine, opiates, PCP, MDMA, methadone) and an alcohol test (breath or urine) will be performed at screening and on Day -1. For Group 4 only, a urine cotinine test will also be performed at screening and on Day -1.

9.10.6 Pregnancy Test

A urine pregnancy test will be performed at screening and at study exit, and a serum pregnancy test will be performed on Day -1.

9.10.7 Laboratory Assessments

Procedures of processing and storing clinical laboratory samples will be detailed in the laboratory manual.

9.10.7.1 Biochemistry

Biochemistry will be performed at screening, on Day -1 and at study exit, following a fasting period of at least 8 hours. The following will be assessed: albumin, alkaline phosphatase, AST, ALT, urea, calcium, chloride, glucose, phosphorus, potassium, creatinine, sodium, total bilirubin, CPK, and total protein.

Serum creatinine will also be measured approximately 24 hours post-dose (Day 2).

9.10.7.2 Cystatin C Measurement

Serum cystatin C will be measured at screening in order to allow, if required, additional analyses based on this endogenous glomerular filtration marker. Only if requested, cystatin GFR will be estimated (eGFR) using the eGFR equation for serum cystatin C. The values obtained will be used for additional data analysis and will have no impact on subjects' eligibility and categorization.

9.10.7.3 Serology

Hepatitis B (HBs Ag), Hepatitis C (HCV) antibody, and HIV antigen and antibody detection will be performed at screening.

9.10.7.4 Genetic Testing

Genetic testing for mitochondrial mutations will be performed at screening.

9.10.7.5 Hematology

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

9.10.7.6 Coagulation

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

9.10.7.7 Urinalysis

Urinalysis will be performed at screening, 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.

9.10.8 Determination of Creatinine Clearance

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.

The corrected CrCl values obtained may be used for additional data analysis.

9.10.9 Renal Injury Biomarkers

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.

9.11 Study Exit / Early Termination Procedures

Study exit procedures are scheduled to be performed at the follow-up visit on Day 8 (±1 day). If not possible, or in case of early termination, all efforts will be made to complete study exit / early termination procedures within 14 days after the last participation of the subject in the study.

The study exit procedures include a brief physical examination, hematology, biochemistry, coagulation, urinalysis, vital signs (BP, HR, RR and OT), 12-lead ECG, urine pregnancy test, injection site evaluation, and AE monitoring.

9.12 Data Collection and Evaluation

All clinical raw data will be recorded promptly, accurately, legibly, and indelibly by the clinical staff on raw data sheets and/or recorded electronically using validated and Code of Federal Regulations (CFR) part 11 compliant software(s) and transcribed into Case Report Forms (CRFs). All raw data will be conserved in order to maintain data integrity. A physician and/or the clinical staff will assume the responsibility of ensuring the completeness and accuracy of the clinical data. Please refer to Section 15 for record retention requirements.

9.13 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 PI 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 the clinical site SOP:

- safety reason;
- non-compliance with protocol requirements;
- significant protocol deviation;
- positive alcohol test, cotinine test (Group 4 only), drug screen, or pregnancy test.

For RI subjects (Groups 1 to 3) taking medications allowed by the PI, 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 PI.

Hematology, biochemistry, urinalysis, and coagulation results will be reviewed by the 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.

Subjects who withdraw or are withdrawn from the study after dosing, for reasons other than safety and tolerability, may be replaced in order to ensure a minimum of 6 completed subjects per group. The total number of subjects dosed (including potential replacement subjects) will remain within a maximum of 8 subjects per group and within a maximum of 32 subjects for the whole study. Additional 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.

9.14 Adverse Events

9.14.1 Recording of Adverse Events

AEs will be recorded and evaluated for their seriousness, severity, and relationship to the study medication. AEs will be collected and documented during the course of the study, from screening up to study exit. AEs will be followed-up until complete resolution, or until a Sub-Investigator judges safe to discontinue follow-up. For events considered related to study drug, follow-up by the Investigator may be required until the event or its sequelae resolve or stabilize at a level acceptable to the Investigator, and Eloxx Pharmaceuticals concurs with that assessment. The relationship to the study medication will be classified according to section 9.14.3.

9.14.2 Assessment of Severity

The severity of AEs will be described and documented using the following definitions:

Table 4. Assessment of AE Severity

Severity	Description
Mild	Awareness of signs and symptoms, but are easily tolerated; are of minor irritant type; causing no limitations of usual activities. Signs or symptoms may require minor action.
Moderate	Discomfort severe enough to cause some limitations of usual activities and may require action.
Severe	Incapacitating with inability to carry out usual activities or significantly affects clinical status, and requires specific action and/or medical attention.

9.14.3 Assessment of Relationship to the Study Drug

Each AE must be classified based on medical judgment and according to the following categories: certain, probable/likely, possible, unlikely, or unrelated.

The definitions for the causality assessments according to the WHO-Uppsala Monitoring Center (UMC) system for standardized case causality assessment are as follows:

Note: all of the assessment criteria per causality should be reasonably complied with.

Certain:

- Event or laboratory test abnormality with plausible time relationship to drug intake;
- Cannot be explained by disease or other drugs;
- Response to withdrawal plausible (pharmacologically, pathologically);
- Event definitive pharmacologically or phenomenologically (i.e., an objective and specific medical disorder or a recognized pharmacological phenomenon);
- Rechallenge satisfactory, if necessary.

Probable/Likely

- Event or laboratory test abnormality with reasonable time relationship to drug intake;
- Unlikely to be attributable to disease or other drugs;
- Response to withdrawal clinically reasonable;
- Rechallenge not required.

Possible

- Event or laboratory test abnormality with reasonable time relationship to drug intake;
- Could also be explained by disease or other drugs;
- Information on drug withdrawal may be lacking or is unclear.

Unlikely

- Event or laboratory test abnormality with a time to drug intake that makes a relationship improbable (but not impossible);
- Disease or other drugs provide plausible explanations.

Unrelated

- It does not follow a reasonable temporal sequence from the administration of the test drug;
- It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject;
- It does not follow a known pattern of response to the test drug.

9.14.4 Serious Adverse Events

9.14.4.1 Definition of Serious Adverse Event

A SAE is any event that meets any of the following criteria:

- Death
- Life-threatening
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

- Congenital anomaly/birth defect in the offspring of a subject who received rimegepant
- Other: Important medical events that may not result in death, be life-threatening, or require hospitalization, may be considered an SAE 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 events are:
  - ✓ Intensive treatment in an emergency room or at home for allergic bronchospasm
  - ✓ Blood dyscrasias or convulsions that do not result in inpatient hospitalization
  - ✓ Development of drug dependency or drug abuse

9.14.4.2 Serious Adverse Event Reporting to the Sponsor

The Investigator or designee must report all serious adverse events (SAEs) [redacted] immediately, or within 24 hours of knowledge by the Investigator or his staff, regardless of the presumed relationship to the study drug. The notification must be directed to:

[redacted]

[redacted] will work with the clinical site to obtain complete information on the event. When further information becomes available, the SAE should be updated with the new information and reported via the same [redacted] contact information.

Any SAE will be reported to the Sponsor by [redacted] via telephone and by fax or e-mail, within 24 hours of [redacted] becoming aware of the event, and then in writing as soon as possible, but no later than 7 calendar days after first knowledge of the SAE. The notification must be directed to:

[redacted]

Must be copied on all notifications:

[redacted]

9.14.4.3 Serious Adverse Event Reporting to Regulatory Agency(ies)

The Sponsor is responsible for notifying the FDA of suspected, unexpected, serious adverse drug reactions observed during conduct of studies in which the investigational drug is administered.

FDA notification of fatal or life-threatening suspected, unexpected, serious adverse drug reaction must be made as soon as possible, but no later than 7 calendar days after becoming aware of the information. FDA notification of all other suspected, unexpected, serious adverse drug reactions

that are neither fatal nor life-threatening must be made as soon as possible, but no later than 15 calendar days after becoming aware of the information.

The Sponsor is responsible to comply with any other applicable regulatory requirement(s) related to the reporting of SAE to other regulatory authority(ies).

9.14.4.4 Serious Adverse Event Reporting to the Independent Ethics Committee

It is the responsibility of each clinical site to report as soon as possible, but no later than 7 calendar days after first knowledge by the Investigator, fatal or life-threatening suspected, unexpected, serious adverse drug reactions occurring at its site to the Independent Ethics Committee (IEC) responsible for the study.

It is the responsibility of each clinical site to report to the IEC all other suspected, unexpected, serious adverse drug reactions that are neither fatal nor life-threatening, as soon as possible, but no later than 14 calendar days after first knowledge by the Investigator.

9.15 On-Study Pregnancy

If a subject or partner of a subject participating in the study becomes pregnant during the study, the Investigator should report the pregnancy to [redacted] within 24 hours of being notified (see contact information in section 9.14.4.2).

A subject becoming pregnant while on study drug will immediately be withdrawn from the study and early termination study procedures will be performed.

The subject or partner should be followed by the Investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the Investigator should notify [redacted] At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for SAE reporting.

[redacted] will report the information related to on-study pregnancies to the Sponsor as per established safety management procedures.

9.16 Reportable Disease

In the case a subject has or manifested any clinical signs characteristic of a reportable disease or condition (e.g., HIV, tuberculosis, SARS), it is the responsibility of the PI to notify the public health department of the [redacted] within 72 hours after becoming aware of the information.

10. Study Termination

The study may be terminated by the PI following consultation with the Sponsor, by the Sponsor or by the regulatory authorities. Following a decision to discontinue the trial, the PI will immediately inform the active study subjects and the IEC responsible for this trial, stating the reasons for discontinuation of the study and, furthermore, advise them in writing of any potential risks to the health of study subjects or other persons. It is the Sponsor's responsibility to report

the premature termination of the study to the regulatory authority(ies), when required by the applicable regulatory requirement(s).

11. Analytical Methodology

Samples will be transported to the bioanalytical facility in at least two separate shipments, with each set of aliquots in separate shipments. Once the bioanalytical laboratory confirms receipt of the first shipment, the second set of aliquots may be sent. The samples should be packed on sufficient dry ice to keep them frozen for at least 72 hours.

All shipments will be accompanied by an inventory list and delivered to the following address:

Attn: Sample Coordination
[redacted]

Clinic personnel will notify the analytical laboratory prior to shipment by phone, fax or e-mail.

[redacted] will analyze ELX-02 in plasma and urine samples using validated methods.

The bioanalytical work in support to the study will be conducted in compliance with the GCP using the SOPs in place in the bioanalytical laboratory and as per applicable regulations.

Samples from subjects included in the PK population (see section 12.2.2) and from subjects who were withdrawn from the study due to AEs will be analyzed.

12. Pharmacokinetic and Statistical Analyses

Pharmacokinetic analysis will be performed using Phoenix® WinNonlin®, which is validated for bioequivalence/bioavailability studies by inVentiv. Inferential statistical analyses will be performed using SAS® according to FDA guidelines.

Interim PK analyses will be performed after completion of dosing in the mild (Group 1) and moderate (Group 2) RI patients.

12.1 Pharmacokinetics

12.1.1 Plasma Pharmacokinetic Parameters

The following PK parameters will be calculated by standard non-compartmental methods with ELX-02 plasma concentrations:

1) 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.
2) AUC_{0-t}: area under the concentration-time curve from time zero to the last non-zero concentration
3) AUC_{0-inf}: area under the concentration-time curve from time zero to infinity (extrapolated)
4) C_{max}: maximum observed concentration
5) Residual area: calculated as $100*(1- AUC_{0-t} / AUC_{0-inf})$
6) T_{max}: time of observed C_{max}
7) $T_{½ el}$: elimination half-life
8) K_{el}: elimination rate constant
9) CL/F: apparent total clearance (L/hr) estimated from dose/AUC_{0-inf}
10) V_d/F: apparent volume of distribution (L) estimated from CL/K_{el} (β)

12.1.2 Urine Pharmacokinetic Parameters

The following PK parameters will be calculated by standard non-compartmental methods with ELX-02 urine concentrations:

1) $Ae_{interval}$: Amount of drug excreted in urine for each time interval, calculated as the urine concentration multiplied by the urine volume.
2) Ae_{0-t}: Cumulative urinary excretion from time zero to time t, calculated as the sum of the amounts excreted over each collection interval.
3) 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.
4) T_{Rmax}: Time of maximal urinary excretion, calculated as the midpoint of the collection interval during which R_{max} occurred.
5) Fe_{0-t}: Fraction (% dose) excreted unchanged
6) CL_R: Renal clearance, calculated as Ae_{0-72} / AUC_{0-72}

Additional pharmacokinetic analysis may be performed.

12.2 Analysis Populations

12.2.1 Safety Population

The safety population is defined as all subjects who received at least one dose of the study medication.

12.2.2 Pharmacokinetic Population

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

Data (concentrations and PK parameters) from subjects withdrawn due to AEs will be presented but excluded from descriptive statistics.

12.3 Statistical Analyses

12.3.1 Statistical Analyses of Pharmacokinetic Parameters

GFR estimated with serum creatinine concentrations and the MDRD4 equation will be used to classify subjects and this classification will serve for primary analyses. If applicable, GFR, PK, and statistical analyses estimated with serum cystatin C concentrations will be presented for supportive purposes.

Individual and mean 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 $AUC_{0\text{-}72}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and C_{max} at the alpha level of 0.05 to compare groups (Mild, Moderate, Severe and Control). Factor incorporated in the model will include Group as a fixed effect. Inter-subject coefficient of variation will be estimated. The ratio of geometric means (Mild/Control, Moderate/Control, Severe/Control) and 90% CI for the ratio of geometric means, based on least-squares means from the ANOVA of the ln-transformed data, will be calculated for $AUC_{0\text{-}72}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and C_{max}. T_{max} will be analyzed nonparametrically with point estimates and 90% CIs for the median differences of T_{max} between study groups (Mild-Control, Moderate-Control, Severe-Control).

For urine PK parameters, using GLM procedures in SAS, ANOVA will be performed on ln-transformed $Ae_{0\text{-}t}$ and R_{max} at the alpha level of 0.05 to compare groups (Mild, Moderate, Severe and Control). Factor incorporated in the model will include Group as a fixed effect. Inter-subject coefficient of variation will be estimated. The ratio of geometric means (Mild/Control, Moderate/Control, Severe/Control) and 90% CI for the ratio of geometric means, based on least-squares means from the ANOVA of the ln-transformed data, will be calculated for $Ae_{0\text{-}t}$ and R_{max}.

Additional statistical analysis may be performed.

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, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and C_{max}) will be determined by a linear or non-linear regression.

12.3.2 Safety and Tolerability Parameters and Analyses

Demographic parameters will be summarized descriptively.

Safety and tolerability to 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, physical examination, vital signs, ECG, early markers of renal injury, and clinical laboratory parameters. TEAEs will be summarized descriptively by renal function group (Mild, Moderate, Severe renal impairment, and Control) for all subjects who were

dosed (safety population). Changes from baseline values in vital signs, ECG, and clinical laboratory parameters will be evaluated. Safety and tolerability data will be reported using descriptive statistics. No inferential statistical analysis of safety data is planned.

Details of statistical analyses will be developed in a Statistical Analysis Plan (SAP).

13. Final Report

Clinical and statistical sections of the report will be the responsibility of inVentiv. The bioanalytical section of the report will be the responsibility of QPS.

In the event that the study is prematurely terminated, inVentiv will produce an abbreviated safety report. In such an event, raw data will not be submitted with the abbreviated report but will be archived at each site, unless requested by the Sponsor.

14. Regulatory Considerations and Quality Assurance

14.1 Independent Ethics Committee Approval of Protocol and Other Study Documents

The Investigators agree to provide the IEC with all appropriate documents, including a copy of the protocol/amendments, ICFs, advertising text (if any), Investigator's Brochure (if any) and any other written information provided to study subjects. The trial will not begin until the Investigators have obtained the IEC favourable written approvals for the above-mentioned study documents. A properly executed written ICF shall be read, signed, and dated by each subject prior to entering the trial or prior to performing any study procedure. The original signed and dated ICF will be kept at the clinical site and a copy will be given to the subject.

In the event that the protocol is amended, the revised protocol must be approved by the IEC prior to its implementation, unless the changes involve only logistical or administrative aspects of the trial. If a revised ICF is introduced during the study, each subject's further consent must be obtained. The new version of the ICF must be approved by the IEC, prior to subsequently obtaining each subject's consent.

The PI and the Sponsor's representative must sign the protocol and its amendments (if any) before initiating the study.

It is the Sponsor's responsibility to submit the protocol and its amendments (if any), and the ICFs to regulatory authorities when necessary.

14.2 Compliance

This study will be conducted in compliance with the protocol, GCP, and all applicable regulations, including the Federal Food, Drug and Cosmetic Act, U.S. applicable Code of Federal Regulations (title 21), and any IEC requirements relative to clinical studies. The study will also be conducted in compliance with the recommendations laid down in the most recent version of the Declaration of Helsinki, with the exception that registration of such Phase 1 trials in a publicly accessible database is not mandatory.

14.3 Liability

If a bodily injury is sustained, resulting directly from the use of the study drug, the Sponsor will reimburse for reasonable physician fees and medical expenses necessary for diagnosis and treatment of only the bodily injury, provided that the injury is not due to a negligent or wrongful act or omission by the study doctor and his/her staff, in which case the clinical site would cover associated fees.

14.4 Quality Assurance Program

The clinical sites have established Quality Control (QC) and Quality Assurance (QA) systems with written SOPs to ensure that the study will be conducted and data will be generated, recorded, and reported in compliance with the protocol, GCP, and applicable regulatory requirements. A rigorous QC program is applied to ensure accuracy of all data and reports.

14.5 Audits, Inspections and Monitoring

In accordance with the principles of GCP and GLP, the study may be inspected by the QA unit of the clinical sites, regulatory authorities, and the Sponsor. The Sponsor is entitled to access information about the status of the study and to review the original documents of the study.

15. Confidentiality and Retention of Essential Documentation

This document contains trade secrets and commercial information that is confidential and may not be disclosed to third parties. Persons to whom this study protocol is disclosed must be informed that all the information herein is confidential and may not be further divulged. These restrictions will apply as well to all future communications if deemed privileged or confidential. Publication of the study results may only be allowed with written permission from the Sponsor.

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

16. References

1 ELX-02 Investigator’s Brochure. Eloxx Pharmaceuticals. Edition no. 3.2, released on 26 June 2018.

2 Gentamicin Injection, USP (Fresenius Kabi USA, LLC), Prescribing Information. Version revised on October 2013. Available online at: http://www.accessdata.fda.gov/scripts/cder/daf/

3 CDER. U.S. FDA, Draft Guidance for Industry: Pharmacokinetics in Patients with Impaired Renal Function - Study Design, Data Analysis, and Impact on Dosing and Labeling. March 2010. Available at: https://www.fda.gov/downloads/Drugs/Guidances/UCM204959.pdf

4 EMA, Guideline on the evaluation of the pharmacokinetics of medicinal products in patients with decreased renal function. EMA/CHMP/83874/2014. December 2015. Available at: https://www.ema.europa.eu/documents/scientific-guideline/guideline-evaluation-pharmacokinetics-medicinal-products-patients-decreased-renal-function_en.pdf

5 Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1. March 2017. Available at: https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables

17. APPENDIX 1

Table 5. 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 *Report only 1*	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* *Report only 1* (> 15 years of age)	2.5 to <5 cm in diameter OR 6.25 to <25 cm^2 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 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social and functional activities	≥10 cm in diameter OR ≥100 cm^2 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 (eg, abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only 1* (> 15 years of age)	2.5 to <5 cm in diameter OR 6.25 to <25 cm^2 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 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social and functional activities	≥10 cm in diameter OR ≥100 cm^2 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 (eg, abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Pruritus	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

* Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area.