Eloxx Pharmaceuticals

A Phase 1, Randomized, Double-Blinded, Placebo-Controlled, Third Party Open, Multiple Dose Escalation Study to Evaluate the Safety, Tolerability and Pharmacokinetics of Subcutaneously Administered ELX-02 in Independent Consecutive Cohorts of Healthy Subjects

Compound:	ELX-02
Compound Name:	6'-(R)-Methyl-5-O-(5-amino-5,6-dideoxy-α-L-talofuranosyl)- paromamine sulfate
European Clinical Trials Database (EudraCT) Number:	2016-005249-21
US IND Number:	137391
Protocol Number:	EL-002
Phase:	1b
Sponsor:	Eloxx Pharmaceuticals. 950 Winter Street Waltham, MA 02451-1208
[redacted]	[redacted]
Protocol Version	29 May 2019

Summary of Changes: Protocol ELX-02 Dated 26 February 2019 to Protocol ELX-02 Dated 29 May 2019

Synopsis and Section 3.2 Progression to Next Cohort Dose Escalation were revised to include additional language regarding dose interruption and resumption during the study related to high frequency threshold shifts.

Signatures

Signature of Sponsor Representative

A Phase 1, Randomized, Double-Blinded, Placebo-Controlled, Third Party Open, Multiple Dose EscalationStudy to Evaluate the Safety, Tolerability and Pharmacokinetics of Subcutaneously Administered ELX-02 in Independent Consecutive Cohorts of Healthy Subjects

[redacted]

'This Clinical Study Protocol has been reviewed and approved by the Sponsor in order to ensure compliance with Good Clinical Practice.'

[redacted]

Signature of Investigator

A Phase 1, Randomized, Double-Blinded, Placebo-Controlled, Third Party Open, Multiple Dose Escalation Study to Evaluate the Safety, Tolerability and Pharmacokinetics of Subcutaneously Administered ELX-02 in Independent Consecutive Cohorts of Healthy Subjects

Name: [redacted]

I declare that I have read and understood this study protocol. I agree to abide by this protocol (subject to any amendments agreed in writing between the Sponsor and Principal Investigator). Any changes in procedure will only be made if necessary to protect the safety, rights, or welfare of the subjects.
This study will be conducted according to Good Clinical Practice (GCP), local regulations and to all stipulations, clinically and administratively, as stated in the protocol, including all statements as to confidentiality. It is agreed that the conduct and results of this study will be kept confidential.
It is agreed that the protocol contains all necessary information required to conduct the study as outlined in the protocol, and that the study will not be initiated without the approval of the Independent Ethics Committee (IEC)/Institutional Review Board (IRB) and the local health autority.

Signature:

Date:

Signature of Investigator

A Phase 1, Randomized, Double-Blinded, Placebo-Controlled, Third Party Open, Multiple Dose Escalation, Study to Evaluate the Safety, Tolerability and Pharmacokinetics of Subcutaneously Administered ELX-02 in Independent Consecutive Cohorts of Healthy Subjects

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Signature:

Date:

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PROTOCOL SUMMARY

Study Title:	A Phase 1, randomized, double-blinded, placebo-controlled, third party open, multiple dose escalation study to evaluate the safety, tolerability and pharmacokinetics of subcutaneously administered ELX-02 in independent consecutive cohorts of healthy subjects.
Protocol No.:	EL-002
Phase	1b
Eudra CT Number	2016-005249-21
IND Number	137391
Test Drug	ELX-02: 6'-(R)-Methyl-5-O-(5-amino-5,6-dideoxy-α-L-talofuranosyl)- paromamine sulfate
Reference Drug	NaCl 0.9%
Study Objectives:	**Primary** 1. To assess the safety and tolerability of multiple ascending subcutaneously (SC) administered doses of ELX-02. 2. To study the pharmacokinetics (PK) of ELX-02 administered as multiple SC doses. **Secondary** 1. To assess whether a maximum tolerated dose (MTD) is attained within the given dose range. 2. To assess linearity between ascending SC doses and PK parameters.
Study Endpoints	**Primary** 1. Incidence and characteristics of adverse events (AEs) and other changes in safety parameters at ascending doses. 2. Blood and urine PK parameters of ELX-02. **Secondary** 1. Assessment, based on the safety profile, whether dose-limiting toxicity (DLT) and MTD are attained within the tested dose range. 2. Dose linearity of PK parameters within the administered SC doses range.
Study Design and Procedures:	This is a Phase 1, randomized, double-blinded, placebo-controlled, multiple dose escalating study in healthy male and female 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 needs 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 will receive ELX-02 and three will receive placebo: • Cohort 1: ELX-02 0.1 mg/kg or placebo SC twice a week for 9 doses; • Cohort 2: ELX-02 0.3 mg/kg or placebo SC twice a week for 9 doses; • Cohort 3: ELX-02 1.0 mg/kg or placebo SC twice a week for 9 doses; • Cohort 4: ELX-02 2.5 mg/kg (100 mg/mL) or placebo SC twice a week for 9 doses; • Cohort 5: ELX-02 up to 2.5 mg/kg (50 mg/mL per injection) or placebo SC twice a week for 9 doses; • Cohort 6: ELX-02 2.5 to 5.0 mg/kg or placebo SC twice a week for 9 doses. • Cohort 7: ELX-02 up to 5.0 mg/kg or placebo SC twice a week for 9 doses. Dose escalation to the next cohort will not be performed until after the 14-day observation period for the previous cohort, to allow for detection of unanticipated delayed adverse event (AE)s. Changes to the planned dose may be made in any cohort, depending on safety and any available PK data. The dose will not exceed 5.0 mg/kg. Additional cohorts or subjects may

	be added to reaffirm safety of ELX-02. The investigational drug, ELX-02, is formulated as a solution for SC injection, containing 200 mg/mL. The placebo control will consist of a SC injection of normal saline at matching volumes. The study will comprise the following periods for each subject: • A screening period, within 42 days before dosing up to Day -2 (including an ear, nose, and throat (ENT) exam with auditory and vestibular assessment (up to Day -8). An additional baseline ENT exam with auditory and vestibular assessment will be performed between Day -7 and Day -1 (may be combined on the same day 2-3 hours apart). • An in-house treatment and follow-up period consisting of a predose day (Day -1), dosing day (Day 1) and follow-up days (Days 2 and 3). Subjects will be released from the Clinical Pharmacological Unit (CPU) approximately 48h after dosing. • Subject will return to the ENT specialist for auditory and vestibular tests on Day 3 (-1 day). • A return visit to the CPU for treatment and testing on Day 4. • An in-house treatment period consisting of a predose day (Day 7) and a dosing day (Day 8) • A return visit for performing audiometric and vestibular tests on Day 10 (-1 day) • A return visit to the CPU for treatment and testing on Day 11. • An in-house treatment period consisting of a predose day (Day 14) and a dosing day (Day 15). • A return visit for performing audiometric and vestibular tests on Day 17 (-1 day). • A return visit to the CPU for treatment and testing on Day 18. • An in-house treatment period consisting of a predose day (Day 21) and a dosing day (Day 22). • A return visit for performing audiometric and vestibular tests on Day 24 (-1 day). • A return visit to the CPU for treatment and testing on Day 25. • An in-house treatment and follow-up period consisting of a pre-last dose day (Day 28), dosing day (Day 29) and follow-up days (Days 30 and 31). Subjects will be released from the CPU approximately 48h after the last dosing. • A return visit to the CPU on Day 32. • An End-of-Study (EOS) visit will take place 7-10 days after last dosing. The additional ENT exam may be performed 7-11 days after last dosing. <u>Dose escalation</u> The decision to proceed to a higher dose level will be following the recommendations of both the general and auditory and vestibular Data Safety Monitoring Boards (DSMB, AVDSMB) based on the dose-escalation stopping rules (see below). The decision will be based on review of the blinded safety data, any available blinded PK data, and the results of audiologic tests and questionnaires collected up to the DSMB meeting. The AVDSMB will provide recommendation to the general DSMB following review of the results of audiologic tests and questionnaires. The next cohort will be dosed after the general DSMB and AVDSMB have evaluated the safety data and any available PK data and have recommended to escalate.

The principles of dose escalation, determination of DLT and MTD, are as follows:

Per Common Terminology Grades for Adverse Events (CTCAE) guidelines,

- Grade-1 drug-related toxicity will allow dose escalation to the next dose level.
- Grade-2 drug-related toxicity:
  - Cohort 1:
    - If the total number of subjects with this drug-related toxicity does not exceed 3, dose escalation is allowed to Cohort 2.
    - If the total number of subjects with this drug-related toxicity is 4 or more, this will be considered a DLT and dose escalation to Cohort 2 will not be authorized.
  - Cohorts 2 and above:
    - If this toxicity occurs in up to 1 subject per cohort, dose escalation to the next dose level is permitted.
    - If this toxicity occurs in 3 subjects per cohort, 6 subjects receiving ELX-02 and 3 subjects receiving placebo will be added to the current dose level:
      - If the number of subjects of the repeated dose level with this drug-related toxicity does not exceed 3, dose escalation is permitted.
      - If 4 or more subjects of the repeated dose level have this drug-related toxicity, this will be considered a DLT and dose escalation to the next dose level will not be authorized. The previous dose level will be determined as the MTD.
- Grade-3, 4 or 5 drug-related toxicity: if it occurs at any dose level and in any cohort, this will be considered a DLT and the previous dose level will be determined as the MTD.

Outside of CTCAE guidelines:

For abnormalities in high frequency audiometry (HFA), an ototoxic event is defined as the loss of >25 dB averaged at three consecutive frequencies in at least one ear.

- For confirmed ototoxic event in HFA in any cohort:
  - If an HFA ototoxic event occurs in 2 subjects receiving ELX-02 in any cohort, 6 subjects receiving ELX-02 and 3 subjects receiving placebo will be added to the current dose level, unless it is determined by the Sponsor with the AVDSMB following a thorough medical review that the study may resume. In this instance additional subjects will not need to be added.
  - If an HFA ototoxic event occurs in ≥3 subjects receiving ELX-02 in any cohort, this will be considered a DLT and dose escalation to the next dose level will not be authorized. The previous dose level will be determined as the MTD.

If the criteria for stopping a dose for an individual subject has been met during a cohort, the study may be temporarily suspended immediately, to allow for further medical evaluation with additional assessments of the proported event, as appropriate. If any subject/subjects meet dose stopping or significant change criteria the sponsor, after discussion with PI, may pause the study for another careful review of data for all subjects; Study may be resumed for the remaining subjects after discussions between the sponsor and site occur, taking into consideration the safety surveillance of the discontinued participants, as well as, the dosed ones.If this occurs, the following process will be initiated:

- Subjects will continue their participation with the same dose in order to assure that all subjects receive their entire complement of nine doses per cohort.
- Subjects who have received the required number of injections for a dose on a dosing day prior to study suspension will continue with their next administration, regardless of the treatment day, in order to receive their total of 9 doses per cohort.
- Subjects who did not receive the required number of injections on a dosing day prior to study suspension will not need to repeat that dosing day, they will proceed to the next administration, regardless of the treatment day, in order to receive their total of nine doses per cohort.

	The discontinued subjects who met the significant change or dose stopping criteria will be followed for safety and surveillance audiology tests; the monitoring period will continue either until the resolution of the event, such time that it is determined that changes are permanent or the subject is lost to follow-up. The determination for permanency will be made by the AVDSMB and does not have a specified time frame. *Note: The number of subjects with a particular drug-related toxicity reflects those with the same preferred term according to the Medical Dictionary for Drug Regulatory Affairs (MedDRA). **Note: toxicity will be rated according to the CTCAE grading rules. For adverse events of interest related to Ear and Labyrinth Disorders, the adjudication committee will assess the grading according to the CTCAE guidelines. ***Note: specific laboratory grading rules will be specified in the protocol for kidney injury molecule (KIM-1) and clusterin based on the laboratory's Normal Ranges for these parameters. ****Note: injection site reactions will be assessed according to the Division of Aids (DAIDS) criteria.
Investigational Product	ELX-02 is a synthetic aminoglycoside formulated as a solution with water for SC injection, containing 200 mg/mL. A 0.9% sodium chloride (NaCl) sterile solution for injection will be used to prepare the appropriate dilutions of ELX-02.
Placebo	Solution for SC injection containing saline (NaCl 0.9%), at matching volumes.
Mode of Administration	• SC injection(s) will be administered to the abdominal region around the umbilicus or to any area with a significant subcutaneous adipose tissue (e.g. thigh).
Safety and Tolerability Assessments	• Adverse events (AE) monitoring • Incidence of events of special interest (hypersensitivity, nephrotoxicity, auditory, and vestibular toxicity) • Local reaction of injection site • Safety lab tests (blood and urine) • Vital signs (supine blood pressure (BP), heart rate (HR), respiratory rate (RR), and oral temperature) • 12-lead electrocardiogram (ECG) taken after 5 minutes in a supine position • Physical examination
Other Assessments	• Concomitant medications • Urine drug screen (UDS) • Seum alcohol test • Blood or urine pregnancy test (all women, regardless of childbearing potential) • Renal injury biomarkers (KIM-1 and clusterin) • Potential read-through of housekeeping proteins
PK Assessments	Blood and urine samples will be collected for ELX-02 determination. The following PK parameters will be determined for ELX-02: C_{max}, $C_{predose}$, C_{1h}, t_{max}, AUC_t, AUC_{24h}, AUC_{48h}, AUC_{72h}, AUC_{inf}, $t_{1/2}$, volume of distribution (Vd/F), clearance (CL/F), accumulation ratio (Rac), and an estimation of ELX-02 dose linearity of PK parameters. Subjects should consume at least 500 mL of water within 120 minutes before dosing. The predose PK blood sample will be taken right before study drug administration. The study staff will encourage the subjects to drink at least one glass of fluids every hour up to 10h after dosing. The following parameters will be calculated based on ELX-02 urine concentrations: urinary excretion (Ae) of ELX-02 (in mass and %dose - fe) for each collection interval, cumulated urinary excretion (in %dose) for each collection interval, total excretion (in %dose) and renal clearance (using the AUC and Ae for the same time interval).

Number of Subjects:	Up to 63 healthy adult subjects will participate in the study: 9 in each cohort randomized 2:1 to ELX-02 and placebo. Both males and females need to be enrolled in each cohort. Additional cohorts or subjects may be added to reaffirm safety of ELX-02.
Inclusion Criteria:	Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study: 1. Be able and willing to provide written Informed Consent indicating that the subject has been informed of all pertinent aspects of the study. 2. Healthy female subjects and male subjects who, at the time of screening, are between the ages of 18 and 55 years, inclusive. Healthy is defined as no clinically relevant abnormalities identified by a detailed medical history, full physical examination, including BP and pulse rate measurement, 12-lead ECG, and clinical laboratory tests. 3. Female subjects of non-childbearing potential must meet at least one of the following criteria: • Achieved postmenopausal status, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; post-menopausal status will be confirmed by a serum follicle-stimulating hormone level; • Have undergone a documented hysterectomy and/or bilateral oophorectomy; • Have medically confirmed ovarian failure. All other female subjects (including females with tubal ligations) will be considered to be of childbearing potential and may be enrolled if they have negative pregnancy tests at screening and admission day and agree to use a highly effective method of contraception for 14 days before first study drug administration and 28 days after last study drug administration. Female subjects of childbearing potential must agree to undergo repeated pregnancy tests. 4. Male subjects must be willing to use an effective method of contraception. They must agree to use a condom consistently and correctly, during the course of the study until 28 days after last study drug administration. 5. Not using any prescription medication and dietary supplements within 30 days or 5 half-lives (whichever is longer) prior to the first study drug administration, except for contraceptives – nor be taking any over-the-counter (OTC) herbal or medicinal products. As an exception, acetaminophen/paracetamol may be used at doses of ≤2 g/day. 6. Non-smoking and no use of any tobacco or nicotine products (by declaration) for a period of at least 6 months prior to screening visit. 7. Be on no medication with potential to impair renal function (e.g., non-steroidal anti-inflammatory [NSAID]s) or with ototoxic potential (e.g., quinine, salicylates, aminoglycosides). 8. Normal renal function (glomerular filtration rate >60 mL/min/1.73m^2) based on creatinine plasma concentration and the Modification of Diet in Renal Disease (MDRD) equation for estimated glomerular filtration rate. Subjects with lower MDRD clearance can be included on the condition that they have a normal 24h creatinine clearance (determined by a 24h urine collection). 9. Negative human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab) serology tests at screening. 10. No history of alcohol or DOA. Negative UDS and serum alcohol test at screening and Day -1. 11. No personal history (or current) or hereditary hearing loss, persistent tinnitus, persistent vertigo, persistent imbalance, persistent unsteadiness and no regular exposure to excessive noise (such as employment in a factory, music teacher, etc). 12. Body Mass Index (BMI) of 19.0 to 32.0 kg/m2 (inclusive); and a total body weight >50.0 kg (110 lbs) and <100.0 kg.

Exclusion Criteria	Subjects with any of the following characteristics/conditions will not be included in the study: 1. Subjects who are investigational site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the Investigator, or subjects who are the Sponsor employees directly involved in the conduct of the study. 2. Concurrent participation or participation in another clinical trial within at least 5 tissue half-lives prior to dosing (calculated from the previous study's last dosing day). If the previous trial involved agents with delayed effects or prolonged metabolism, a 12 months interval is required. 3. Evidence or history of clinically relevant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease (including drug allergies). 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 Investigator, would make the subject inappropriate for entry into this study. 4. Presence of mitochondrial mutations making subject susceptible to aminoglycoside toxicity. 5. Subjects with any history of ear disease or surgeries, persistent dizziness or persistent tinnitus. 6. Subjects with any abnormality at screening, that indicates the presence of a vestibular pathology, conductive hearing loss or balance problem (by an ENT). Subjects with audiometry abnormalities in the conventional frequencies (up to 8 kHz) results at screening as follows: any pure-tone threshold >55 dB and/or inter-ear difference in any frequency of >20 dB. Dizziness Handicap Inventory (DHI)-H score >16. Tinnitus Handicap Inventory (THI)-H score >14. 7. History of regular alcohol consumption exceeding 14 drinks/week for females or 21 drinks/week for males (1 drink = 150 mL of wine or 360 mL of beer or 45 mL of hard liquor) within 6 months of screening. 8. Screening supine BP ≥140 mm Hg (systolic) or ≥90 mm Hg (diastolic), following at least 5 min of supine rest. If BP is ≥140 mm Hg (systolic) or ≥90 mm Hg (diastolic), the BP should be repeated two more times and the average of the three BP values should be used to determine the subject's eligibility. 9. Screening supine (for 5 minutes) 12-lead ECG demonstrating QTc >450 msec for men and >470 msec for women, or a QRS interval >120 msec. If QTc or QRS exceed these limits, the ECG should be repeated two more times and the average of the three QTc or QRS values should be used to determine the subject's eligibility. 10. Subjects with **ANY** abnormalities in clinical laboratory tests at screening, considered by the study physician as clinically relevant. In particular, subjects with alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine and total bilirubin ≥1.5 upper limit of normal will be excluded. 11. Pregnant or breastfeeding female subjects; 12. Subjects who donated blood or received blood or plasma derivatives in the three months preceding study drug administration. 13. Unwilling or unable to comply with all scheduled visits, treatment plan, laboratory tests and other study procedures and the restrictions described in this protocol. 14. Known relevant allergy to any drug and/or aminoglycosides. 15. Subjects with an inability to communicate well with the Investigators and CPU staff (e.g., language problem, poor mental development).

	16. Subjects with visual impairment or inability to read and comprehend the DHI and THI scales. 17. Subjects with any acute medical situation (e.g., acute infection) within 48 hours (h) of study start, which is considered of significance by the Investigator.
Statistical Methods	No formal sample size was estimated. The number of 9 subjects included in each dose group is deemed sufficient to respond to exploratory safety/tolerability and PK purposes. Statistical analyses will be performed using SAS® v9.2 or higher (SAS Institute, Cary NC, United States of America [USA]), PK parameters calculation will be performed using Phoenix 6.2 (Pharsite Corp., USA) or later Adverse events will be classified by system-organ classes and preferred terms and then summarized by number and percentage of volunteers experiencing AEs. Pharmacokinetic parameters of ELX-02 (C_{max}, t_{max}, AUC_t, AUC_{24h}, AUC_{48h}, AUC_{72h}, AUC_{inf}, $t_{1/2}$, Vd/F, and CL/F) will be summarized by descriptive statistics by dose group and day of administration (sample size, mean, median, standard deviation, CV%, minimum and maximum, geometric mean and geometric CV%, when applicable). Steady-state achievement will be evaluated by visual inspection of predose (and 1h postdose) concentrations figures. The following parameters will be calculated based on ELX-02 urine concentrations: Ae of ELX-02 (in mass and %dose - fe) for each collection interval, cumulated urinary excretion (in %dose) for each collection interval, total excretion (in %dose) and renal clearance (using the AUC and Ae for the same time interval). For each of the PK parameters C_{max} and AUCs, the dose linearity will be assessed using a power model, including the log-transformed PK parameters as dependent variables and the log-transformed dose, day and dose*day as fixed effects. The slope for log-transformed dose (ß) will be estimated with its 90% confidence interval to examine dose proportionality. The t_{max} will be compared between dose groups and days using the appropriate non-parametric tests. Descriptive statistics will be used to summarize demographics, baseline characteristics, and safety data. Aggregate study data will be analyzed from all cohorts for the study report, regardless of where the cohort was conducted.
Duration of Clinical Phase	Approximately 12 weeks for each subject including a screening period of up to 6 weeks before first dosing, 4 weeks after the first dose and 2 weeks for final safety follow-up.

Table 1: Time and Events Schedule

Day in Study	Hours Postdose	Informed consent[a]	Demographics	Medical history[b]	Inclusion/exclusion criteria	Weight	Height, body mass index	Vital signs[d]	Physical examination[e]	Blood safety lab tests[f]	General urinalysis	HIV, HbsAg, HCVAb	Blood pregnancy + FSH (females)	Urine pregnancy (females)[g]	Urine drug screen (UDS)	Serum alcohol test	12-lead ECG[h]	Audiometric testing[i]	Vestibular Questionnaires (DHI, THI)	Confinement to CPU[j]	Discharge from CPU (2 hr after administration)	Blood PK[k]	Urine collection for PK[l]	Urine collection for creatinine[m]	**Dosing**[n]	Local reaction of injection site	Urine for renal injury biomarkers[m]	Blood samples for retention[o]	Blood for housekeeping proteins	Sample for mitochondrial mutations testing	AE and CM monitoring
Screen Day -42 to -2		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[i]	X					X			X			X	X
Day -1					X	X			X	X[q]	X			X	X	X				X				X			X				X
Day 1	**-180 min**				X	X[c]		X									X					X	X	X			X	X	X	X	X
	0																								**X**						X
	15 min																					X	0-12 h								X
	30 min							X														X									X
	45 min							X														X				X					X
	1h							X														X									X
	3h							X									X					X					X				X
	6h								X	X[q]	X						X					X				X					X
	12h							X									X					X	12-24 h	X		X	X				X

Day in Study	Hours Postdose	Informed consent[a]	Demographics	Medical history[b]	Inclusion/exclusion criteria	Weight	Height, body mass index	Vital signs[d]	Physical examination[e]	Blood safety lab tests[f]	General urinalysis	HIV, HbsAg, HCVAb	Blood pregnancy + FSH (females)	Urine pregnancy (females)[g]	Urine drug screen (UDS)	Serum alcohol test	12-lead ECG[h]	Audiometric testing[i]	Vestibular Questionnaires (DHI, THI)	Confinement to CPU[j]	Discharge from CPU (2 hr after administration)	Blood PK[k]	Urine collection for PK[l]	Urine collection for creatinine[m]	**Dosing**[n]	Local reaction of injection site	Urine for renal injury biomarkers[m]	Blood samples for retention[o]	Blood for housekeeping proteins	Sample for mitochondrial mutations testing	AE and CM monitoring
Day 2	**24h**							X		X[q]	X						X					X	24-48h	X		X	X		X		X
	36h																					X		X			X				X
Day 3	**48 h**					X		X	X	X[q]	X						X	X[p]	X[p]		X	X		X		X	X		X		X
Day 4						X[c]		X														X	48-72h	X	**X**	X	X				X
Day 7						X				X[q]	X			X	X	x				X											X
Day 8						X[c]		X	X								X					X		X	**X**	X	X	X	X		X
Day 10						X												X[p]	X[p]												
Day 11						X		X														X		X	**X**	X	X				X
Day 14						X				X[q]	X			X	X	x				X											X
Day 15						X[c]		X	X								X				X	X		X	**X**	X	X	X	X		X
Day 17						X												X[p]	X[p]												
Day 18						X		X														X		X	**X**	X	X				X
Day 21						X				X[q]	X			X	x	x				X											X

Day in Study	Hours Postdose	Informed consent[a]	Demographics	Medical history[b]	Inclusion/exclusion criteria	Weight	Height, body mass index	Vital signs[d]	Physical examination[e]	Blood safety lab tests[f]	General urinalysis	HIV, HbsAg, HCVAb	Blood pregnancy + FSH (females)	Urine pregnancy (females)[g]	Urine drug screen (UDS)	Serum alcohol test	12-lead ECG[h]	Audiometric testing[i]	Vestibular Questionnaires (DHI, THI)	Confinement to CPU[j]	Discharge from CPU (2 hr after administration)	Blood PK[k]	Urine collection for PK[l]	Urine collection for creatinine[m]	**Dosing**[n]	Local reaction of injection site	Urine for renal injury biomarkers[m]	Blood samples for retention[o]	Blood for housekeeping proteins	Sample for mitochondrial mutations testing	AE and CM monitoring
Day 22						X[c]		X	X								X				X	X		X	**X**	X	X	X	X		X
Day 24						X												X[p]	X[p]		X										
Day 25						X		X														X		X	**X**	X	X				X
Day 28						X		X	X	X[q]	X			X	X	X				X				X			X				X
Day 29	**-180 min**					X[c]		X									X					X	X	X			X	X	X		X
	0																								**X**						X
	15 min																					X	0-12 h								X
	30 min							X														X									X
	45 min							X														X				X					X
	1 h							X														X									X
	3h							X									X					X						X			X
	6h								X	X[q]	X						X					X				X					X
	12h							X									X					X	12-24 h	X		X	X				X

Day in Study	Hours Postdose	Informed consent[a]	Demographics	Medical history[b]	Inclusion/exclusion criteria	Weight	Height, body mass index	Vital signs[d]	Physical examination[e]	Blood safety lab tests[f]	General urinalysis	HIV, HbsAg, HCVAb	Blood pregnancy + FSH (females)	Urine pregnancy (females)[g]	Urine drug screen (UDS)	Serum alcohol test	12-lead ECG[h]	Audiometric testing[i]	Vestibular Questionnaires (DHI, THI)	Confinement to CPU[j]	Discharge from CPU (2 hr after administration)	Blood PK[k]	Urine collection for PK[l]	Urine collection for creatinine[m]	**Dosing**[n]	Local reaction of injection site	Urine for renal injury biomarkers[m]	Blood samples for retention[o]	Blood for housekeeping proteins	Sample for mitochondrial mutations testing	AE and CM monitoring
Day 30	**24h**							X		X[q]	X						X					X	24-48h	X		X	X		X		X
	36h																					X		X			X				X
Day 31	**48h**							X	X	X[q]	X						X				X	X		X		X	X		X		X
Day 32	**72h**																					X	48-72h	X			X				
Day 36								X	X	X[q]	X	X		X		X	X	X	X					X		X	X		X		X

AE: Adverse event, CM: Concomitant therapy, HIV: Human Immunodeficiency Virus, HbsAg: Hepatitis B surface antigen, HCVAb: Hepatitis C virus antibody, CPU: Clinical Pharmacological Unit, DHI: Dizziness Handicap Inventory, THI: Tinnitis Handicap Inventory.

a. Informed consent to be provided before any study-related assessment.
b. Including hearing and balance disorders.
c. Weight only to be taken if not already done the previous day.
d. Vital signs include heart rate (HR), blood pressure (BP), respiratory rate (RR), and oral body temperature. Vital parameters will be assessed in supine position after at least 5 min. supine rest.
e. A complete physical examination is to be performed at all timepoints except for the 6h postdose assessment on Day 1 and Day 29 when a brief examination is to be performed.
f. Biochemistry, hematology, and coagulation. All blood samples for safety assessments should be taken after an overnight fast of at least 8h, except for the sample taken at 6h postdose on Day 1 and Day 29. At screening, subjects will be instructed not to eat within 3h before arrival at the CPU.

g. Urine pregnancy tests only to be performed for women regardless of childbearing potential.
h. A single 12-lead ECG will be taken providing QT, QTc, HR, QRS, and PR after 5 minutes supine.
i. Subjects will be scheduled for audiometric and vestibular tests preceded by an ENT physical exam in a specialized department. The auditory and vestibular testing will be performed at Screening (to confirm eligibility), and prior to initial dosing to determine a baseline and will include: (1) Otoscopy, (2) Immittance audiometry (commonly called tympanometry), (3) Speech Reception Threshold, (4) Pure Tone Audiometry (PTA) with frequencies up to 8 kHz if possible (should there be a PTA threshold of ≥15dB, the subject should undergo bone conduction testing), (5) High frequency audiometry (HFA) with frequencies up to 16 kHz if possible, (6) Tinnitus Handicap Inventory, (7) Dizziness Handicap Inventory. Follow up testing will be performed at Day 3 (-1 day), Day 10 (-2 days), Day 17 (-2 days), Day 24 (-2 days), and EOS (7-11 days after last dosing). The otoscopic exam on Day 3, Day 10, Day 17 and Day 24 can be performed 72 hours prior to dosing by a CPU physician.
j. Subjects will come to the CPU for confinement on Day -1, Day 7, Day 14, Day 21, and Day 28.
k. The predose PK blood sample will be taken in a standardized manner for all subjects, right before study drug administration.
l. During the 0-12h collection period urine will be collected every hour, during the 12-24h collection period every 6h (i.e., 12-18h collection and 18-24h collection), and during the 24-48h collection period urine will be collected every 12h (i.e., 24-36h collection and 36-48h collection). Urine gravity will be calculated for each collection. The subjects will collect the urine at home (in urine jars) during the 48h-72h collection period.
m. Creatinine samples will be taken at all timepoints corresponding to renal injury biomarker collection. On days 1-4 and 29-32, samples for renal injury biomarkers and creatinine will be taken from urine spot samples at 24hr, 36hr, 48hr and 72hr post dose.
n. Subjects should consume at least 500 mL of water within 120 minutes before dosing. Subcutaneous (SC) injection (s) in the abdominal wall as per the subject's body weight. The study staff will encourage the subjects to drink at least one glass (approximately 250 mL) of fluids every hour up to 10h after dosing.
o. All blood samples for retention need to be taken after an overnight fast of at least 8h, except for the samples taken at 3h postdose on Day 1 and Day 29.
p. Subject will return to the ENT specialist for auditory and vestibular tests within 48 hours prior to dosing. The otoscopic exam on Day 3, Day 10, Day 17 and Day 24 can be performed 72 hours prior to dosing by a CPU physician.
q. No GFR/MDRD sample/analysis beyond the screening period.

ABBREVIATIONS

Abbreviation	Term
AAA	American Academy of Audiology
AE	Adverse events
Ae	Cumulative amount of unchanged drug excreted into urine
AEOI	Adverse event of interest
ALT	Alanine aminotransferase (also known as glutamate-pyruvate transaminase-SGPT)
ASHA	American Speech-Language-Hearing Association
AST	Aspartate aminotransferase (also known as glutamate-oxaloacetate transaminase-SGOT)
AUC_t	Area under the plasma concentration-time curve calculated from time of administration to the last quantifiable concentration, computed using the linear trapezoidal rule
AUC_{inf}	Area under the concentration-time curve from time 0 extrapolated to infinity
AUC_{24h}	Area under the plasma concentration-time curve calculated from time of administration to time 24h, computed using the linear trapezoidal rule
AUC_{48h}	Area under the plasma concentration-time curve calculated from time of administration to time 48h (after first dose only), computed using the linear trapezoidal rule
AUC_{72h}	Area under the plasma concentration-time curve calculated from time of administration to time 72h, computed using the linear trapezoidal rule
AVDSMB	Auditory and Vestibular Data Safety Monitoring Board
BMI	Body mass index
BP	Blood pressure
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CL/F	Apparent plasma clearance
C_{max}	Maximum plasma concentration
CNS	Central nervous system
$C_{predose}$	Trough plasma concentration observed at the end of the dosing interval (i.e. at each predose starting from second dose)
CPU	Clinical Pharmacological Unit
CRF	Case report form
CSA	Clinical study agreement
CTA	Clinical trial application
CTCAE	Common Terminology Grades for Adverse Events

Abbreviation	Term
DAIDS	Division of Aids
DHI	Dizziness Handicap Inventory
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic acid
DOA	Drugs of abuse
DSMB	Data Safety Monitoring Board
EDMC	External Data Monitoring Committee
EDP	Exposure during pregnancy
ECG	Electrocardiogram
ENT	Ear nose and throat
EOS	End of study
EU	European
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GAG	Glycosaminoglycans
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HbsAg	Hepatitis B surface antigen
HCT	Hematocrit
HCV Ab	Hepatitis C virus antibody
HED	Human Equivalent Dose
HFA	High Frequency Audiometry
HIV	Human Immunodeficiency Virus
HR	Heart rate
h,	Hour, hours
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	Intravenous
K	Potassium
kg	Kilogram
KIM-1	Kidney injury molecule-1
LLOQ	Lower Limit of Quantification
LPLV	Last visit of the last subject in the study

Abbreviation	Term
LS	Life Sciences
MA	Medical Affairs
MDRD	Modification of Diet in Renal Disease
MPS	Mucoplysaccharidose type I
min	Minute
mL	Milliliter
mmHg	Millimeter mercury
MRSD	Maximum recommended starting dose
MRT	Mean residence time
Mecp2	Methyl-CpG-binding protein 2
msec	Millisecond
MTD	Maximum tolerated dose
Na	Sodium
NOAEL	No observed adverse effect level
ºC	Degrees centigrade
NSAIDs	Non-steroidal anti-inflammatory drugs
OTC	Over the counter
PI	Principal investigator
PK	Pharmacokinetics
PTA	Pure Tone Audiometry
Rac	Accumulation ratio
RBC	Red blood cells
RNA	Ribonucleic acid
rRNA	Ribosome Ribonucleic acid
RR	Respiratory Rate
SAE	Serious adverse event
SC	Subcutaneous
SRT	Speech Reception Threshold
SUSAR	Suspected unexpected serious adverse reaction
$t_{½}$	Elimination half-life
THI	Tinnitus Handicap Inventory
t_{max}	Time at which C_{max} occurs
tRNA	Transfer Ribonucleic acid
USA	United States of America
UDS	Urine drug screen
Vd/F	Apparent volume of distribution
versus	vs

Abbreviation	Term
WBC	White Blood Cell

STUDY ADMINISTRATIVE STRUCTURE

SPONSOR	Eloxx Pharmaceuticals. 950 Winter Street Waltham, MA 02451-1208 United States
Sponsor Medical Director:	[redacted]
Senior Director, Clinical Operations:	[redacted]
BIOANALYTICAL LABORATORY	[redacted]
Project Leader:	[redacted]
LABORATORY FOR GENETIC TESTING	[redacted]
Project Leader	[redacted]
LABORATORY FOR RETENTION OF BLOOD SAMPLES	[redacted]
Project Leader	[redacted]
LABORATORY FOR SAFETY LABS	[redacted]

1. INTRODUCTION

1.1. Mechanism of Action/Indication

1.1.1. The Effect of Aminoglycosides on Nonsense Mutations

Aminoglycosides bind to the decoding site in the small subunit of the ribosome RNA (rRNA)[1] that normally monitors proper codon-anticodon interactions and can induce translational read-through. When aminoglycosides bind to the decoding site, they induce a conformational change that reduces the ability of rRNA to discriminate between cognate and near-cognate aminoacyl-transfer(t)RNAs[2] and stabilize 3 critical adenosine residues in a conformation suitable for read-through[3]. This reduction in the accuracy of codon-anticodon recognition increases the probability that translational read-through of stop codons occurs. Despite promising results, aminoglycosides use as a long-term TRID therapy is restricted since their antibiotic activity may damage the microflora and lead to resistance against pathogenic bacteria. In addition, prolonged use of aminoglycosides is associated with ototoxicity and/or nephrotoxicity[4, 5, 6].

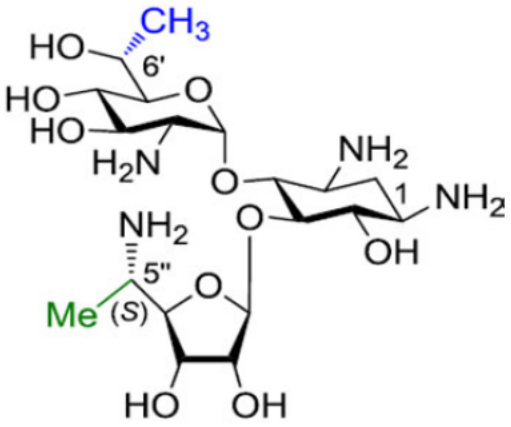
ELX-02 was optimized through successive rounds of medicinal chemistry that took into account an in depth understanding of the prokaryotic ribosome crystal structure[7], the role of the prokaryotic residue A1408 versus (vs) its counterpart eukaryotic residue G1408, the function of residues A1492, and A1493 in the interaction of aminoglycosides to the ribosomal A site[8, 9, 10, 3].

Rational design separated the structural elements of the aminoglycoside scaffold inducing read-through from those elements affecting antibacterial activity[11, 12]. [redacted] ELX-02 interacts selectively with the eukaryotic ribosome G1408 residue, through the 5' amine group of the 3rd ring stabilizing the flipping out of the two conserved alanine residues in the rRNA caused by binding of cognate tRNAs[3].

1.1.2. ELX-02

ELX-02 is a [redacted] drug, containing an aminoglycoside-based scaffold [6'-(R)-Methyl-5-O-(5-amino-5,6-dideoxy-α-L-talofuranosyl)-paromamine sulfate]. Its chemical structure is shown in Figure 1:

Figure 1: Chemical Structure of ELX-02



ELX-02 is a designer aminoglycoside with unique pharmacological properties.

Like classical aminoglycosides, ELX-02 has poor oral bioavailability (Investigators' Brochure[19]) but rapid parenteral (subcutaneous [SC], intravenous [IV]) absorption, reaches peak plasma concentrations within 30-60 minutes (min) after parenteral administration, has poor protein binding, has facilitated penetration in the renal cortex and low penetration into pharmacologically privileged compartments such as the central nervous system (CNS) and cochlea, is not metabolized, and is excreted unchanged by the kidney via glomerular filtration in direct proportion to creatinine clearance, and has concentration dependent activity whereby clearance depends on renal function and volume of distribution depends on weight. More details can be found in the Investigators' Brochure[19].

The pharmacokinetic profile of ELX-02 in plasma in animals and humans shows dose proportionality and follows classic multi-exponential decay with a rapid and large elimination phase and a slower and low distribution phase. [redacted]

[redacted]

1.2. Background and Rationale

Nonsense mutations generating premature termination codons account for approximately one third of all genetic diseases. Nonsense mutation is a genetic mutation in a deoxyribonucleic acid (DNA) sequence that results in a shorter, unfinished protein product. During protein formation, a DNA (or RNA) codon, comprising a three nucleotides sequence, corresponds to a specific amino acid or stop signal (stop codon). Nonsense codons do not code for an amino acid, but instead, together with a complex mechanism of translation termination at the 3i three prime untranslated region (UTR) region, including several release factors, signal the end of protein synthesis. Thus, nonsense mutations occur when a premature nonsense is introduced in the DNA sequence. When the mutated sequence is translated into a protein, the resulting protein is incomplete and shorter than normal. Consequently, most nonsense mutations result in nonfunctional proteins[13, 14].

Examples for genetic disease where nonsense mutations are part of their phenotype:

- Rett syndrome: an X-linked postnatal severe and disabling neurodevelopmental disorder characterized by loss of communication skills, epilepsy, motor and tone disorder, sleep and behavioral difficulties and autonomic dysfunction primarily abnormal breathing patterns[15].
- Mucopolysaccharidosis type I (Hurler syndrome): is a genetic disorder that results in the buildup of glycosaminoglycans (GAG) (formerly known as mucopolysaccharides) due to a deficiency of alpha-L iduronidase. The condition is marked by progressive deterioration, hepatosplenomegaly, dwarfism and unique facial features. There is a progressive mental retardation, with death frequently occurring by the age of 10 years[16].
- Duchenne muscular dystrophy: a genetic disorder characterized by progressive loss of muscle strength and integrity. Boys affected by this disorder suffer from progressive muscle atrophy and weakness with significant motor impairment. They lose independent ambulation by the age of 13 years and die in their second or third decade[17].
- Cystic fibrosis: a relatively common genetic disease that involves multiple organ systems but chiefly results in chronic respiratory infections, pancreatic enzyme insufficiency, and associated complications in untreated patients[18].

1.2.1. *Non-Clinical Studies*

The translational read-through capabilities and efficacy of ELX-02 have been evaluated in in vitro cellular models and in in vivo animal models of genetic disease caused by nonsense mutations. These models include Cystic Fibrosis (CF), Cystinosis, Duchenne Muscular

Dystrophy (DMD), mucoplysaccharidose type I (MPS I), and Rett syndrome. [redacted]

For additional details of the nonclinical studies, please consult the IB[19].

1.2.2. *Clinical Studies*

Eloxx Pharmaceuticals has completed two monocentric, randomized, double-blinded placebo-controlled single ascending dose studies in human volunteers. These SAD studies evaluated single doses of ELX-02 between 0.3 mg/kg and 7.5 mg/kg in 60 normal volunteers and characterized general and specialized safety parameters, and pharmacokinetics. ELX-02 was generally well tolerated, showed typical pharmacokinetic parameters for an aminoglycoside, and showed an acceptable safety profile without severe or serious drug-related adverse event (AE)s.

Safety

Over dose ranges tested, ELX-02 was shown to be safe and generally well tolerated. No SAEs were reported during the study. For the pooled data of both studies, the most common TEAEs occurring in ≥3 subjects were as follows: injection site reactions (6 subjects [10%]), headache (5 subjects [8%]), injection site erythema (3 subjects [5%]), and ear discomfort (3 subjects

[5%]). All of these TEAEs were considered mild in severity except for one case of moderate headache. One subject in the 5.0 mg/kg SC dose group presented high frequency audiometry (HFA) fluctuations which were deemed due to technical errors and therefore not considered as TEAE. An ENT expert report analyzed auditory and vestibular assessments results and concluded that no signs of ototoxicity were observed.

Pharmacokinetics

ELX-02 given SC was rapidly absorbed with a median time to maximum concentration (t_{max}) of about 0.5-1.0h. $AUC_{0\text{-}inf}$ changed linearly with ELX-02 dose (24-fold increase for a 25-fold dose increase) and C_{max} changed in a quasi proportional way with ELX-02 dose as significant $Dose^2$ effects were seen (17-fold increase for a 25-fold increase). The apparent terminal half-life was 2 to 4h in the dose range tested (0.3 mg/kg to 7.5 mg./kg).

Plasma concentration profiles were linearly and proportionally related to the dose administered. ELX-02 had a rapid elimination phase and a slower distribution phase. ELX-02 was excreted and recovered almost quantitatively in the urine during the first 12 hours.

1.2.3. *Overall Rationale for the Study*

Study Rationale

Eloxx Pharmaceuticals is developing ELX-02 as a treatment for inherited conditions caused by nonsense mutations whereby the mutation leads to decreased or absent protein expression. ELX-02 read-through the nonsense mutation and by that abrogates the effects of nonsense mutations and induces the translation of full-length functional proteins and thus have a beneficial clinical effects in these diseases. Comprehensive preclinical testing of ELX-02 in vitro and in animal models of disease demonstrated its efficacy and decreased toxicity compared to traditional aminoglycosides. Thus, ELX-02 has the potential to treat any disease caused by nonsense mutations and thus have broad applicability for many orphan diseases.
This study is initiated to assess the safety, tolerability, and PK of ELX-02 in healthy subjects, receiving multiple escalating doses as SC injection(s), in a placebo-controlled design. No increase in doses will be made unless the former one has been shown to be safe and tolerable as evaluated by the Data Safety Monitoring Board (DSMB).

Dose Selection Rationale

[redacted]

[redacted]

1.2.4. *Risk Benefit Analysis*

[redacted]

Complete information for this compound may be found in the IB[19].

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objective

1. To assess the safety and tolerability of multiple ascending subcutaneously (SC) administered doses of ELX-02.

2. To study the PK of ELX-02 administered as multiple SC doses.

2.1.2. Secondary Objectives

1. To assess whether a maximum tolerated dose (MTD) is attained within the given dose range.

2. To assess linearity between ascending SC doses and PK parameters.

2.2. Endpoints

2.2.1. Primary Endpoints

1. Incidence and characteristics of AEs and other changes in safety parameters at ascending doses.
2. Blood and urine PK parameters of ELX-02.

2.2.2. Secondary Endpoints

1. Assessment, based on the safety profile, whether dose-limiting toxicity (DLT) and MTD are attained within the tested ELX-02 doses range.
2. Dose linearity of PK parameters within the administered SC doses range.

3. STUDY DESIGN

3.1. Overall Study Design

This is a Phase 1, randomized, double-blinded, placebo-controlled, third party open, multiple dose escalation study to evaluate the safety, tolerability and PK of subcutaneously administered ELX-02 in independent consecutive cohorts of healthy subjects.

The investigational drug, ELX-02, is formulated as a solution for SC injection, containing 200 mg/mL. The placebo control will consist of a SC injection of normal saline at matching volumes.

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 needs 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 will receive ELX-02 and three will receive placebo:

Cohort 1: ELX-02 0.1 mg/kg or placebo SC twice a week for 9 doses;

Cohort 2: ELX-02 0.3 mg/kg or placebo SC twice a week for 9 doses;

Cohort 3: ELX-02 1.0 mg/kg or placebo SC twice a week for 9 doses;

Cohort 4: ELX-02 2.5 mg/kg (100 mg/mL) or placebo SC twice a week for 9 doses;

Cohort 5: ELX-02 up to 2.5 mg/kg (50 mg/mL) or placebo SC twice a week for 9 doses;

Cohort 6: ELX-02 2.5 to 5.0 mg/kg or placebo SC twice a week for 9 doses.

Cohort 7: ELX-02 up to 5.0 mg/kg or placebo SC twice a week for 9 doses.

Dose escalation to the next cohort will not be performed until after the 14-day observation period for the previous cohort, to allow for detection of unanticipated delayed AEs.

Changes to the planned dose may be made in any cohort, depending on safety and any available PK data. The dose will not exceed 5.0 mg/kg.

The assessments performed are summarized per visit in Table 1.

The study will comprise the following periods for each subject:

- A screening period, within 42 days before dosing up to Day -2 (including an ear, nose, and throat (ENT) exam with auditory and vestibular assessment up to Day -8).
    - An additional baseline ENT exam with auditory and vestibular assessment will be performed between Day -7 and Day -1. These assessments (screening and baseline) can be done on the same day (between Day -7 and Day -1) with 2-3 hrs between the 2 assessments.
- An in-house treatment and follow-up period consisting of a predose day (Day -1), dosing day (Day 1), and follow-up days (Days 2 and 3). Subjects will be released from the Clinical Pharmacological Unit (CPU) approximately 48h after dosing.
- Subject will return to the ENT specialist for auditory and vestibular tests on Day 3 (-1 day).
- A return visit to the CPU for treatment and testing on Day 4.
- An in-house treatment period consisting of a predose day (Day 7) and a dosing day (Day 8).

- A return visit for performing audiometric and vestibular tests on Day 10 (-1 day).
- A return visit to the CPU for treatment and testing on Day 11.
- An in-house treatment period consisting of a predose day (Day 14) and a dosing day (Day 15).
- A return visit for performing audiometric and vestibular tests on Day 17 (-1 day).
- A return visit to the CPU for treatment and testing on Day 18.
- An in-house treatment period consisting of a predose day (Day 21) and a dosing day (Day 22).
- A return visit for performing audiometric and vestibular tests on Day 24 (-1 day).
- A return visit to the CPU for treatment and testing on Day 25.
- An in-house treatment and follow-up period consisting of a pre-last dose day (Day 28), dosing day (Day 29), and follow-up days (Days 30 and 31). Subjects will be released from the CPU approximately 48h after the last dosing.
- A return visit to the CPU on Day 32.
- An End-of-Study (EOS) visit will take place 7-10 days after last dosing. The additional ENT exam may be performed 7-11 days after last dosing.

The duration will be approximately 12 weeks for each subject including a screening period of up to 6 weeks before first dosing, 4 weeks after the first dose and 2 weeks for final safety follow-up.

3.2. Progression to Next Cohort and Dose Escalation

The decision to proceed to a higher dose level will be following the recommendations of both the general and auditory and vestibular DSMBs based on the dose-escalation stopping rules (see below). The decision will be based on review of the blinded safety data, any available blinded PK data, and the results of audiologic tests and questionnaires collected up to the DSMB meeting and the recommendations of the AVDSMB following review of the results of audiologic tests and questionnaires. The next cohort will be dosed after the DSMB and AVDSMB have evaluated the safety data and any available PK data and have recommended to escalate.

More details on the DSMB are provided in Section 8.16.

The principles of dose escalation, determination of DLT and MTD, are as follows:

For CTCAE related events,

- Grade-1 drug-related toxicity will allow dose escalation to the next dose level.
- Grade-2 drug-related toxicity:
    - Cohort 1:

      If the total number of subjects with this drug-related toxicity does not exceed 3, dose escalation is allowed to Cohort 2.

      If the total number of subjects with this drug-related toxicity is 4 or more, this

will be considered a DLT and dose escalation to Cohort 2 will not be authorized.

- Cohorts 2 and above:

  If this toxicity occurs in up to 1 subject per cohort, dose escalation to the next dose level is permitted.

  If this toxicity occurs in 3 subjects per cohort, 6 subjects receiving ELX-02 and 3 subjects receiving placebo will be added to the current dose level:

  If the number of subjects of the repeated dose level with this drug-related toxicity does not exceed 3, dose escalation is permitted.

  If 4 or more subjects of the repeated dose level - have this drug-related toxicity, this will be considered a DLT and dose escalation to the next dose level will not be authorized. The previous dose level will be determined as the MTD.

- Grade-3, 4 or 5 drug-related toxicity: if it occurs at any dose level and in any cohort, this will be considered a DLT and the previous dose level will be determined as the MTD.

Outside of CTCAE guidelines:

For abnormalities in HFA, an ototoxic event is defined as the loss of >25 dB averaged at in three consecutive frequencies in at least one ear.

- For a confirmed ototoxic event in HFA in any cohort:
  - If an HFA ototoxic event occurs in 2 subjects receiving ELX-02 in any cohort, 6 subjects receiving ELX-02 and 3 subjects receiving placebo will be added to the current dose level, unless it is determined by the Sponsor and the AVDSMB, following a thorough medical review, that the study may resume. In this instance additional subjects will not need to be added.
  - If an HFA ototoxic event occurs in ≥3 subjects receiving ELX-02 in any cohort, this will be considered a DLT and dose escalation to the next dose level will not be authorized. The previous dose level will be determined as the MTD.

If the criteria for stopping a dose has been met for an individual subject during a cohort, the study may be temporarily suspended immediately to allow for further medical evaluation with additional assessments of the proported event, as appropriate. If any subject/subjects meet dose stopping or significant change criteria, the sponsor, after discussion with PI, may pause the study for another careful review of data for all subjects; Study may be resumed for the remaining subjects after discussions between the sponsor and site, taking into consideration the safety surveillance of the discontinued participants, as well as, the dosed ones. If this occurs, the following process will be initiated:

- Subjects will continue their participation with the same dose in order to assure that all subjects receive their entire complement of nine doses per cohort.
- Subjects who have received the required number of injections for a dose on a dosing day prior to study suspension will continue with their next administration, regardless of the treatment day, in order to receive their total of 9 doses per cohort.
- Subjects who did not receive the required number of injections on a dosing day prior to study suspension will not need to repeat that dosing day, they will proceed to the next administration, regardless of the treatment day, in order to receive their total of

nine doses per cohort.

The discontinued subjects who met the significant change or dose stopping criteria will be followed for safety and surveillance audiology tests; the monitoring period will continue either until the resolution of the event, such time that it is determined that changes are permanent or the subject is lost to follow-up. The determination for permanency will be made by the AVDSMB and does not have a specified time frame.

*Note: The number of subjects with a particular drug-related toxicity reflects those with the same preferred term according to the Medical Dictionary for Drug Regulatory Affairs (MedDRA).

**Note: toxicity will be rated according to the Common Terminology Grades for Adverse Events (CTCAE)[21] grading rules. For adverse events of interest related to Ear and Labyrinth disorders, the adjudication committee will assess the grading.

***Note: specific laboratory grading rules will be specified in the protocol for Kidney injury molecule (KIM-1) and clusterin based on the laboratory's Normal Ranges for these parameters.

****Note: injection site reactions will be assessed according to the Division of Aids (DAIDS) criteria (Section 13.1).

The subsequent cohorts will be dosed at least 14 days following last dosing of the previous cohort.

Figure 2: Study Design Scheme

Cohort 1	ELX-02 0.1 mg/kg or placebo SC twice a week for 9 doses
≥14 days following last dosing of the previous cohort and following advice DSMB and AVDSMB	
Cohort 2	ELX-02 0.3 mg/kg or placebo SC twice a week for 9 doses
≥14 days following last dosing of the previous cohort and following advice DSMB and AVDSMB	
Cohort 3	ELX-02 1.0 mg/kg or placebo SC twice a week for 9 doses
≥14 days following last dosing of the previous cohort and following advice DSMB and AVDSMB	
Cohort 4	ELX-02 2.5 mg/kg (100 mg/mL) or placebo SC twice a week for 9 doses
≥14 days following last dosing of the previous cohort and following advice DSMB and AVDSMB	
Cohort 5	ELX-02 up to 2.5 mg/kg (50 mg/mL per injection) or placebo SC twice a week for 9 doses
≥14 days following last dosing of the previous cohort and following advice DSMB and AVDSMB	
Cohort 6	ELX-02 2.5 to 5.0 mg/kg or placebo SC twice a week for 9 doses
≥14 days following last dosing of the previous cohort and following advice DSMB and AVDSMB	
Cohort 7	ELX-02 up to 5.0 mg/kg or placebo SC twice a week for 9 doses.

4. SUBJECT SELECTION

Up to 63 healthy adult subjects will participate in the study at multiple centers: 9 in each cohort randomized 2:1 to ELX-02 and placebo. Both males and females need to be enrolled in each cohort.

This study can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom participation in the study is considered appropriate. All relevant medical and nonmedical conditions should be taken into consideration when deciding whether a particular subject is suitable for this protocol.

Subject eligibility should be reviewed and documented by an appropriate member of the Investigator's study team before subjects are included in the study.

4.1. Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Be able and willing to provide written Informed Consent indicating that the subject has been informed of all pertinent aspects of the study.
2. Healthy female subjects and male subjects who, at the time of screening, are between the ages of 18 and 55 years, inclusive.

   Healthy is defined as no clinically relevant abnormalities identified by a detailed medical history, full physical examination, including blood pressure (BP) and pulse rate measurement, 12-lead electrocardiogram (ECG), and clinical laboratory tests.
3. Female subjects of non-childbearing potential must meet at least one of the following criteria:

   - Achieved postmenopausal status, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; post-menopausal status will be confirmed by a serum follicle-stimulating hormone level;
   - Have undergone a documented hysterectomy and/or bilateral oophorectomy;
   - Have medically confirmed ovarian failure.

   All other female subjects (including females with tubal ligations) will be considered to be of childbearing potential and may be enrolled if they have negative pregnancy tests at screening and admission day and agree to use 2 highly effective methods of contraception for 14 days before first study drug administration and 28 days after last study drug administration. Female subjects of childbearing potential must agree to undergo repeated pregnancy tests. For highly effective methods of contraception see Section 6.5.1.
4. Male subjects must be willing to use 2 highly effective methods of contraception. They must agree to use a condom consistently and correctly, during the course of the study until 28 days after last study drug administration.
5. Not using any prescription medication and dietary supplements within 30 days or 5 half-lives (whichever is longer) prior to the first study drug administration, except for

contraceptives – nor be taking any over-the-counter (OTC) herbal or medicinal products. As an exception, acetaminophen/paracetamol may be used at doses of ≤2 g/day.

6. Non-smoking and no use of any tobacco or nicotine products (by declaration) for a period of at least 6 months prior to screening visit.
7. Be on no medication with potential to impair renal function (e.g., non-steroidal anti-inflammatory [NSAID]s) or with ototoxic potential (e.g., quinine, salicylates, aminoglycosides).
8. Normal renal function (glomerular filtration rate >60 mL/min/1.73m^2) based on creatinine plasma concentration and the Modification of Diet in Renal Disease (MDRD) equation for estimated glomerular filtration rate. Subjects with lower MDRD clearance can be included on the condition that they have a normal 24h creatinine clearance (determined by a 24h urine collection).
9. Negative human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab) serology tests at screening.
10. No history of alcohol or DOA. Negative UDS and serum alcohol test at screening and Day -1.
11. No personal history (or current) or hereditary hearing loss, persistent tinnitus, persistent vertigo, persistent imbalance, persistent unsteadiness and no regular exposure to excessive noise (such as employment in a factory, music teacher, etc).
12. Body Mass Index (BMI) of 19.0 to 32.0 kg/m2 (inclusive); and a total body weight >50.0 kg (110 lbs) and <100.0 kg.

4.2. Exclusion Criteria

Subjects with any of the following characteristics/conditions will not be included in the study:

1. Subjects who are investigational site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the Investigator, or subjects who are the Sponsor employees directly involved in the conduct of the study.
2. Concurrent participation or participation in another clinical trial within at least 5 tissue half-lives prior to dosing (calculated from the previous study's last dosing day). If the previous trial involved agents with delayed effects or prolonged metabolism, a 12 months interval is required.
3. Evidence or history of clinically relevant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease (including drug allergies). 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 Investigator, would make the subject inappropriate for entry into this study.
4. Presence of mitochondrial mutations making subject susceptible to aminoglycoside toxicity.

5. Subjects with any history of ear disease or surgeries, persistent dizziness or persistent tinnitus.
6. Subjects with any abnormality at screening, that indicates the presence of a vestibular pathology, conductive hearing loss or balance problem (by an ENT).

   Subjects with auditory abnormalities in conventional frequencies at screening as follows (up to 8 kHz): any pure-tone threshold >55 dB and/or inter-ear difference in any frequency of >20 dB.

   Dizziness Handicap Inventory (DHI)-H score >16. Tinnitus Handicap Inventory (THI)-H score >14.
7. History of regular alcohol consumption exceeding 14 drinks/week for females or 21 drinks/week for males (1 drink = 150 mL of wine or 360 mL of beer or 45 mL of hard liquor) within 6 months of screening.
8. Screening supine BP ≥140 mm Hg (systolic) or ≥90 mm Hg (diastolic), following at least 5 min of supine rest. If BP is ≥140 mm Hg (systolic) or ≥90 mm Hg (diastolic), the BP should be repeated two more times and the average of the three BP values should be used to determine the subject's eligibility.
9. Screening supine (for 5 minutes) 12-lead ECG demonstrating QTc >450 msec for men and >470 msec for women, or a QRS interval >120 msec. If QTc or QRS exceed these limits, the ECG should be repeated two more times and the average of the three QTc or QRS values should be used to determine the subject's eligibility.
10. Subjects with **ANY** abnormalities in clinical laboratory tests at screening, considered by the study physician as clinically relevant. In particular, subjects with alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine and total bilirubin ≥1.5 upper limit of normal will be excluded.
11. Pregnant or breastfeeding female subjects.
12. Subjects who donated blood or received blood or plasma derivatives in the three months preceding study drug administration.
13. Unwilling or unable to comply with all scheduled visits, treatment plan, laboratory tests and other study procedures and the restrictions described in this protocol.
14. Known relevant allergy to any drug and/or aminoglycosides.
15. Subjects with an inability to communicate well with the Investigators and CPU staff (e.g., language problem, poor mental development).
16. Subjects with visual impairment or inability to read and comprehend the DHI and THI scales.
17. Subjects with any acute medical situation (e.g., acute infection) within 48 hours (h) of study start, which is considered of significance by the Investigator.

4.3. Prohibitions

The subjects must consume at least 500 mL of water within 120 minutes before dosing. The study staff will encourage subjects to drink at least one glass of fluids every hour up to 10h after dosing.

During confinement at the CPU, no food intake in addition to the standard meals and snacks provided at the CPU will be allowed.

Authorization to leave the CPU will be given by the Investigator.

No blood donation will be allowed until 4 weeks after the last study drug administration.

Female subjects of childbearing potential and non-vasectomized male subjects having a female partner of childbearing potential must agree to the use 2 effective methods of contraception throughout the study (for female subjects from 14 days before first study drug administration) and until 28 days after the last administration of study drugs, as outlined in Section 4.4. For details on the existing data regarding the reproductive toxicity, please see the current Investigator Brochure[19].

Information on prohibited therapies can be found in Section 6.1.

4.4. Lifestyle Guidelines

All male subjects who are able to father children and female subjects who are of childbearing potential and are sexually active and at risk for pregnancy must agree to use 2 highly effective methods of contraception consistently and correctly for the duration of the active treatment period (for female subjects from 14 days before first study drug administration) and for at least 28 days after the last dose of investigational product.

Subjects need to affirm that they meet the criteria for correct use of at least 2 of the selected methods of contraception. The Investigator or his/her designee will discuss with the subject the need to use highly effective contraception consistently and document such conversation in the subject's chart. In addition, the Investigator or his or her designee will instruct the subject to call immediately if the selected contraception method is discontinued or if pregnancy is known or suspected in the subject or the subject's partner.

More details on contraception methods for female and male subjects can be found in Section 6.5.1 and Section 6.5.2, respectively.

4.5. Sponsor's Qualified Medical Personnel

The contact information for the sponsor's appropriately qualified medical personnel for the study is documented in the study administrative structure.

4.6. Contact Cards

To facilitate access to appropriately qualified medical personnel on study-related medical questions or problems, subjects are provided with a contact card. The contact card contains, at a minimum, protocol and investigational compound identifiers, subject study numbers, and contact information for the investigational site.

4.7. Rater Qualifications

Audiometric and vestibular tests will be performed by an ENT specialist. More details are provided in Table 1 and in Section 7.3.6.

4.8. Subject Identification

Each subject who has signed the informed consent will be identified by a unique study screening number. Subjects who are dosed will receive an additional Randomization number.

Subject identification will be confirmed by the study staff at each study visit.

4.9. Re-Screening of Subjects

If dosing is not expected to occur within 42 days of initial screening procedure, a re-screening visit will be performed to allow subject's participation. Mitochondrial mutations testings will not need to be repeated.

Subjects who completed all screening procedures and were found eligible to participate in the study may serve as standbys in their designated cohort. In case a standby subject is not dosed, he will be discharged from the CPU but may be invited to participate in another cohort later on.

4.10. Screening Failures

Subjects who fail to meet the entrance criteria at any stage during the screening period are defined as screen failures. All screen failures will be recorded in the Identification and screening logs. The reason(s) for screen failure will be documented. The screening log will be kept in the Investigators Site File.

Screen failure subjects will be withdrawn from the study and will not count towards the total enrolled or total eligible subjects.

4.11. Removal, Replacement or Early Withdrawal

Subjects are free to discontinue their participation in the study at any time and without prejudice to further treatment. The Investigator must withdraw any subject from the study if that subject requests to be withdrawn, or if it is determined that continuing in the study would result in a safety risk to the subject.

Subjects discontinued or withdrawn from the study after receiving ≤6 doses may be replaced upon request from the Sponsor. The subject's participation in this study may be discontinued due to the following reasons:

1. Request by regulatory agency, Sponsor, primary care physician or Investigator.
2. Subject withdraws consent.
3. Female subject is pregnant.
4. Positive UDS and/or serum alcohol test on admission to the CPU on Day -1. If subjects have a positive UDS and/or serum alcohol test after Day -1, there continued participation will be re-evaluated on a case by case basis.
5. Adverse event (AE) or Serious Adverse Event (SAE) meeting criteria for drug-related event and meeting stopping criteria.
6. Subject is unwilling or unable to continue the study or is lost-to-follow-up.
7. Subject is non-compliant with study procedures/study protocol.
8. Investigator decides that withdrawal from the study is in the best interest of the subject.

9. Subject needs medication not allowed in the protocol.
10. Any clinically significant change in subject's medical condition.
11. Subjects may be replaced at the discretion of the Sponsor.

4.12. Early Study Termination

The study may be discontinued prematurely at any time and for any reason. Such reasons may be any of, but not limited to, the following:

1. Occurrence of SAEs of an unanticipated nature in accordance with the stopping rules, severity, and duration or the unexpected incidence of known SAEs (SUSAR);
2. Medical or ethical reasons affecting the continued performance of the study
3. Poor performance or compliance of the site.
4. Exposure of the subjects to unacceptable health risks.
5. A regulatory authority decision.
6. Change in opinion of the IEC.
7. Following DSMB/AVDSMB recommendation.
8. ELX-02 safety concerns.
9. At the discretion of the Sponsor (e.g. decision of the Sponsor to discontinue development of ELX-02 at any time).

If a study is prematurely terminated or discontinued, the Sponsor will promptly notify the PI. After notification, the PI must contact all participating subjects and the site pharmacy within 48 hours. As directed by the Sponsor, all study materials must be collected and the database must be completed to the greatest extent possible.

5. STUDY TREATMENTS

For the purposes of this study, and per International Council for Harmonisation (ICH) guidelines investigational product is defined as a pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use (ICH E6 1.33).

5.1. Allocation to Treatment

Eligible subjects will be assigned to one of the following administrations:

- Cohort 1: ELX-02 0.1 mg/kg or placebo SC twice a week for 9 doses;
- Cohort 2: ELX-02 0.3 mg/kg or placebo SC twice a week for 9 doses;
- Cohort 3: ELX-02 1.0 mg/kg or placebo SC twice a week for 9 doses;
- Cohort 4: ELX-02 2.5 mg/kg (100 mg/mL) or placebo SC twice a week for 9 doses;
- Cohort 5: ELX-02 up to 2.5 mg/kg (50 mg/mL) or placebo SC twice a week for 9 doses;
- Cohort 6: ELX-02 2.5 to 5.0 mg/kg or placebo SC twice a week for 9 doses.
- Cohort 7: ELX-02 up to 5.0 mg/kg or placebo SC twice a week for 9 doses.

Dose escalation to the next cohort will not be performed until after the 14-day observation period for the previous cohort, to allow for detection of unanticipated delayed AEs.

Changes to the planned dose may be made in any cohort, depending on safety and any available PK data. The dose will not exceed 5.0 mg/kg. Additional cohorts and subjects may be added to affirm safety at any dose level, at or below the maximum proposed dose level of 5.0 mg/kg for the study.

The investigational drug, ELX-02, is formulated as a solution for SC injection, containing 200 mg/mL. The placebo control will consist of a SC injection of normal saline at matching volumes.

As subjects are confirmed to be eligible for the study, they will be assigned a single unique identifier across the study.

5.1.1. Randomization and Blinding

At screening, subjects receive a unique screening number. Subjects who are rescreened will be assigned the same screening number they received at first screening but with an additional identifier. Subjects who fail screening and are not enrolled will not be included in the database. The failure details will be documented in the subjects' recruitment file.

Subjects will be assigned to one of the treatment groups. Allocation of each subject to a given treatment group will be described in a randomization list prepared prior to study start by the CRO using SAS® software (SAS Institute Inc., Cary, NC, United States of America [USA]).

The randomization will be balanced using randomly permuted blocks across the treatment groups.

Based on this randomization code, the study drug will be packaged and labeled. Medication code numbers will be preprinted on the study drug labels and assigned as subjects qualify for the study and are randomly assigned to treatment or the pharmacist or appropriate qualified member of the study staff, who is unblinded to treatment and assigned by the PI, will prepare the study drug that corresponds to the assigned subject randomization number.

Blinding will be achieved by the double-dummy method with placebo identical in appearance.

The randomization list will be retained by the CRO until the end of the study (database lock). One copy of the randomization list will be sent in a sealed envelope to the site pharmacist before the start of the study. Another copy will also be sent before the start of the study in a sealed envelope to the bioanalytical laboratory responsible for plasma and urine drug determination. Upon completion of the study, the monitor needs to check the completeness and status of the envelopes and once this is done the monitor can retrieve the envelopes and have them destroyed.

One copy of code breaking envelopes will be made available to the CPU, to be used only in case of emergency. An additional copy of code breaking envelopes will be kept at the CRO. Upon request, the CRO will provide the code-break information to the CRO for SAE reporting purposes. Should a subject experience an AE for which it is necessary to break the blind during the study in order to determine the appropriate treatment for the event, the PI or designee will call the medical monitor prior to unblinding. The reason for unblinding will be documented in the electronic source data (eSource) system. Subjects who are unblinded for any reason during their participation in the study will not be replaced and will be withdrawn immediately from the study.

5.1.2. Breaking the Blind

This will be a double-blinded study; neither the subject nor the Investigators, the study staff who are administering the test products and any personnel involved in subjects' assessment and monitoring, will know the treatment assignment. Only the pharmacists responsible for preparing the clinical test material and the study un-blinded monitor responsible for accountability and, if required, the DSMB members will be un-blinded.

The pharmacy at the CPU will prepare ELX-02/Placebo dose, according to instructions provided in the study's pharmacy manual, based on a prescription issued by the Investigator. Treatment allocation will be available to the pharmacy staff in advance. The study drug will be appropriately labeled, indicating the date and randomization number.

The Sponsor is permitted to request unblinding of any subject in association with adverse events. In the absence of a medical emergency, the blinded randomization for this trial will not be revealed until all data are entered into the database, edits checks are performed, queries closed, CRF signed by the PI, and the database is officially locked.

5.2. Subject Compliance

ELX-02 or placebo will be administered by the appropriately designated study staff at the investigational site, under supervision of the Investigator.

5.3. Investigational Product Supplies

ELX-02 will be prepared in accordance with Good Manufacturing Practice (GMP) as required by the current Good Clinical Practice (GCP).

ELX-02 or Placebo will be labeled according to local law and regulatory requirements. The labels will contain the protocol number, reference number, storage caution statements, dosing instructions, and expiry date.

[redacted]

Eloxx Pharmaceuticals
950 Winter Street
Waltham, MA
02451-1208
United States

5.3.1. Dosage Form(s) and Packaging

[redacted]

PLACEBO: The placebo is a standard 0.9% sodium chloride (NaCl) sterile solution for injection which will be provided by the site pharmacy.

[redacted]

ELX-02 is manufactured and packaged in compliance with the Good Manufacturing Practice (GMP) of drugs, Annex 13 for drugs used in clinical trials.
For any additional Study Drug related information, refer to the Pharmacy Manual.

The syringues will be covered so that no differences in color can be apparent. Detailed instruction will be provided in the pharmacy manual.

5.4. Administration

ELX-02 and matching placebo will be administered to each subject, while confined to bed, by a designated study nurse or Investigator. An Investigator must be present during all ELX-02/Placebo administrations.

Subjects should consume at least 500 mL of water within 120 minutes before dosing.

The drug will be administered as SC injection(s) to the abdominal region around the umbilicus or to any area with a significant subcutaneous adipose tissue (e.g. thigh). Injections for each subject should be performed sequentially to minimize time lag between injections. Detailed instructions on number of injections and injected volume will be specified in the pharmacy manual.

The study staff will encourage the subjects to drink at least one glass (approximately 250 mL) of fluids every hour up to 10h after dosing.

Details of each ELX-02 or matching placebo administration will be recorded.

The used vials from which the drug was administered to the subjects will be retained for dose confirmation. For details on the Study Drug Accountability, refer to the pharmacy manual.

5.5. Investigational Product Storage

[redacted]

Regular temperature logging of the study drug storage freezer at the CPU should be performed. In case a deviation in storage conditions should occur, the CPU must not further dispense the affected study drug and notify the Sponsor.

The Investigator, or an approved representative, e.g., pharmacist, will ensure that all investigational products are stored in a secured area with controlled access under required storage conditions and in accordance with applicable regulatory requirements.

Investigational product should be stored in its original container and in accordance with the label. Site systems must be capable of measuring and documenting (for example, via a log), at a minimum, daily minimum and maximum temperatures for all site storage locations (as applicable, including frozen, refrigerated and/or room temperature products). This should be captured from the time of investigational product receipt throughout the study. Even for continuous monitoring systems, a log or site procedure that ensures active daily evaluation for excursions should be available. The operation of the temperature monitoring device and storage unit (for example, refrigerator), as applicable, should be regularly inspected to ensure it is maintained in working order.

Any excursions from the product label storage conditions should be reported upon discovery. The site should actively pursue options for returning the product to the storage conditions as described in the labeling, as soon as possible. Deviations from the storage requirements, including any actions taken, must be documented and reported to the sponsor. Once an excursion is identified, the investigational product must be quarantined and not used until the sponsor provides documentation of permission to use the investigational product. It will not be considered a protocol deviation if the sponsor approves the use of the investigational product after the temperature excursion. Use of the investigational product prior to sponsor approval will be considered a protocol deviation.

Specific details regarding information the site should report for each excursion will be provided to the site.

5.6. Investigational Product Accountability

The investigative site must maintain adequate records documenting the receipt, use, loss, or other disposition of the investigational product supplies.

The Sponsor's designated site monitor will periodically check the supplies of study drugs held by the Investigator or pharmacist to ensure accountability and appropriate storage conditions of all study drugs used.

Unused study drugs must be available for verification by the site monitor during on-site monitoring visits. Any discrepancies between returned and expected returned study drugs should be explained and documented. All used vials must be stored frozen until the end of the study.

After the last visit of the last subject in the study (LPLV), any unused study drug will be returned to the Sponsor or destroyed at the CPU with the Sponsor's written permission (in this case a certificate of destruction will be provided and filed in the electronic Trial Master File (eTMF).

Hazardous materials such as used ampoules, needles, syringes and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for drug accountability purposes.

5.7. Destruction of Investigational Product Supplies

The sponsor or designee will provide guidance on the destruction of unused investigational product (eg, at the site). If destruction is authorized to take place at the study site, the Investigator must ensure that the materials are destroyed in compliance with applicable environmental regulations, institutional policy, and any special instructions provided by Eloxx Pharmaceuticals, and all destruction must be adequately documented.

6. STUDY RESTRICTIONS

6.1. Concomitant Treatments

There is no knowledge of cross reaction between ELX-02 and other drugs.

Use of prescription or nonprescription drugs and dietary supplements within 30 days or 5 half-lives (whichever is longer) prior to the administration of study drug is prohibited. As an exception,
acetaminophen/paracetamol may be used at doses of ≤2 g/day and contraception (see section 6.5.1.). Herbal supplements should be
discontinued at least 30 days prior to administration of the study drug.

Since ELX-02 is an aminoglycoside derivative, special care should be placed in avoiding concomitant medications which interact with aminoglycosides such as (Gentamicin Product Monograph, 2012):

- Antimicrobials (such as other aminoglycosides, cephalosporins, clindamycin, polymyxin B, clindamycin) which may increase the risk of nephrotoxicity and/or neuro- or ototoxicity;
- Cholinergic agents (e.g., neostigmine, pyridostigmine): Gentamicin antagonizes the effect of neostigmine and pyridostigmine;
- Loop diuretics (e.g., furosemide) which increase the risk for ototoxic and nephrotoxic effects of aminoglycosides;
- Neuromuscular blocking agents and opioid-analgesics (e.g,. fentanyl, succinylcholine);
- Anti-neoplastic agents (e.g., carboplatin, cisplatin) which increase the risk of nephrotoxicity and/or neurotoxicity;
- Immunosuppresive agents (e.g., cyclosporine, tacrolimus) which increase the risk of nephrotoxicity and/or neurotoxicity;
- Mannitol which increases the risk of nephrotoxicity and/or neurotoxicity;
- Magnesium which may increase neuromuscular blockade;
- Quinine – an agents with ototoxic potential;
- Salicylates: agents with ototoxic potential;
- Aminoglycosides;
- Non-steroidal anti-inflammatory drugs (NSAIDs) which may decrease glomerular filtration rate.

If intake of a concomitant drug should become necessary for any reason during the course of the study, the subject is required to inform the Investigator immediately, who will record the drug name, its dose and the time of intake.

6.2. Limit of Noise Exposure

All subjects will be instructed to minimize excessive noise exposure from screening until the completion of the final ENT exams.

In addition, subjects will be required to wear silicone earplugs when exposed to louder than normal noise. However, this will NOT permit them to attend loud concerts, visit shooting range, and so on.

6.3. Meals and Dietary Restrictions

- Subjects shall receive standardized meals while staying in-house at the CPU.
- At least 500 mL water will be consumed within 120 minutes before dosing. The study staff will encourage subjects to drink at least one glass (approximately 250 mL) of fluids every hour up to 10h after dosing. Non-caffeinated drinks may be consumed *ad libitum*.
- Food and drinks containing xanthines (coffee, chocolate, tea etc.) will not be permitted from 48h before first study drug administration until 48h after last administration.
- Subjects will abstain from alcohol from 48h before first study drug administration and during the dosing period until 48h after last study drug administration.
- Subjects will abstain from smoking for 6 months prior to the first screening visit until 48h after the last study drug administration.
- Subjects will abstain from illicit drug use from the first screening visit until 48h after the last study drug administration.

6.4. Activity

Subjects will remain in bed for the first 4 hours (h) after dosing on Day 1 and Day 29 (except when required to void). After this they may be ambulatory but should not engage in strenuous activities (e.g., heavy lifting, weight training, calisthenics, aerobics) until the End of Study (EOS) visit.

6.5. Contraception

All male subjects who are able to father children and female subjects who are of childbearing potential and are sexually active and at risk for pregnancy must agree to use 2 highly effective methods of contraception consistently and correctly throughout the study (from 14 days before first study drug dosing for female subjects) and until 28 days after last study drug administration. The Investigator or designee, in consultation with the subject, will confirm that the subject has selected an appropriate method of contraception from the permitted list of contraception methods (see below). In addition, the Investigator or designee will instruct the subject to call immediately if the selected contraception method is discontinued or if pregnancy is known or suspected in the subject or the subject's partner. All this will be documented in the subject's source documents.

6.5.1. Female Subjects

Female subjects of non-childbearing potential must meet at least one of the following criteria:

- Achieved postmenopausal status, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; post-menopausal status will be confirmed by a serum follicle-stimulating hormone (FSH) level;
- Have undergone a documented hysterectomy and/or bilateral oophorectomy;

- Have medically confirmed ovarian failure.

All female subjects, regardless of childbearing potential, must agree to undergo pregnancy test at screening and on D -1, D7, D14, D21 and D28.

Unless willing to abstain from sexual intercourse for 14 days prior to the first study drug administration, and for 28 days after the last dose, all other female subjects (including females with tubal ligations) will be considered to be of childbearing potential and must use 2 highly effective methods of contraception for two weeks (14 days) before first study drug administration and four weeks (28 days) after last dosing. These include:

- Established use of oral, inserted, injected, implanted or transdermal hormonal methods of contraception (provided the subject plans to remain on the same treatment throughout the entire study and has been using that hormonal contraceptive for an adequate period of time to ensure effectiveness).
- Copper -containing intrauterine device (IUD).

If the female subject has a vasectomized partner (with absence of sperm in the postvasectomy ejaculate), no additional method of contraception is needed.

6.5.2. Male Subjects

All sexually active male subjects will be advised of the potential risks involved in transfer of and exposure to ELX-02 through semen to their partners. Unless they are abstaining from sexual intercourse for 14 days prior to the first study drug administration, and for 28 days after the last dose they must agree to use 2 highly effective method of contraception during the course of the study until 28 days after last dosing. These include a condom, having undergone a vasectomy with absence of sperm in the postvasectomy ejaculate, or having a female partner who meets the criteria for non-childbearing potential.

7. STUDY PROCEDURES

7.1. Timing of Assessments

The timing of assessments is specified below and in Table 1. Allowed time window deviations are provided in Section 7.2.

On days when vital signs and a 12-lead ECG parameters need to be assessed as well as blood sampling, the vital signs and 12-lead ECG will be performed before blood sampling, except when an indwelling cannula has been placed.

Screening Period (Visit 1)

This visit will take place within 42 to 2 days before dosing. Subjects will sign an informed consent form and will be assessed for their eligibility to participate in the study.

The following screening assessments will be performed for each subject:

- Medical history (including questions relating to hearing and balance disorders).
- Contraception method.
- Demographics.
- Physical examination.
- Height, Weight, and BMI (calculated as kg/m^2).
- Vital signs: Supine (following at least 5 min of supine rest) BP, heart rate (HR), respiratory rate (RR) and oral body temperature.
- 12-lead ECG taken after 5 minutes in a supine position.
- Blood and urine lab tests (see Section 7.3.9 for more details):
    - Blood and urine safety tests
    - Serology (HIV, HBsAg, HCVAb)
    - Urine drug screen (UDS)
    - Serum alcohol test
    - Blood pregnancy test
    - FSH
    - Urine sample for creatinine and renal injury biomarker testing
    - Blood sample for mitochondrial mutations testing
- Compliance with inclusion/exclusion criteria.
- Assessment of AEs and concomitant medications.

Screening Visit 2: Auditory and Vestibular Assessments

During the screening period subjects will undergo auditory and vestibular assessments to confirm eligibility (Day -42 to -8). In addition, a baseline exam (PTA and HFA will be duplicated) will be performed prior to initial dosing (within 7 days prior to dose). These assessments can be done on the same day (between Day -7 and Day -1) with 2-3 hrs between the 2 assessments.

The auditory tests to be performed are outlined in Section 7.3.6.

In addition, subjects will complete vestibular and tinnitus questionnaires.

Only subjects having normal test results according to an ENT physician may participate in the study.

Treatment Period

Day -1

Subjects will come to the CPU on Day -1 and will be interviewed by study personnel regarding concomitant medications and any change in their health status or diet since screening. Inclusion/exclusion criteria will be reconfirmed and eligible subjects will undergo the following procedures:

- Physical examination.
- Weight.
- Blood and urine safety tests (see Section 7.3.9 for more details).

The subjects will stay confined till the morning of Day 3.

The following assessments will be done :

- UDS.
- Urine pregnancy test (all women regardless of childbearing potential).
- Serum alcohol test.
- Urine sample for creatinine and renal injury biomarker testing (see Section 7.5.1).
- Assessment of AEs and concomitant medications.

Day 1

Predose

Inclusion/exclusion criteria will be reconfirmed.

Prior to drug administration, the following activities will be completed:

- First morning urine sample for PK determination, for creatinine and renal injury biomarkers assessment.
- Vital signs (supine, BP, HR, RR, and oral temperature) after at least 5 min supine.
- 12-lead ECG taken after 5 minutes in a supine position
- Assessment of AEs and concomitant medications.

- Weight (if not yet taken on Day -1).
- Subjects will drink at least 500 mL water within 120 minutes before dosing.
- Blood samples for PK, housekeeping proteins, and retention (a retention sample will be taken to assess any potential safety issues e.g., hematology and biochemistry, coagulation) will be drawn. The PK blood sample will be taken 180 min before dosing.

Study Drug administration

Each subject will receive ELX-02 or Placebo with SC injection as per subject's body weight measured at Day -1 or Day 1 predose, while confined to bed, as described in Section 5.4. Date and time of drug administration will be documented.

Postdose

- After dosing, subjects will remain confined to bed for four hours (except when required to void). They will then be allowed to move freely within the ward but must refrain from strenuous physical activity for the duration of the study.
- Blood samples for PK will be collected at 15 min, 30 min, 45 min, 1h, 3h, 6h, and 12h postdose (Section 7.4).
- Vital signs (supine BP, HR, RR, and oral temperature) will be measured at 30 min, 45 min, 1h, 3h, and 12h postdose after 5 minutes in a supine position.
- Brief physical examination at 6h postdose.
- Blood and urine safety test at 6h postdose.
- Urine collection for PK during 0-12h hourly UC (each void collected separately) and 12-24h postdose, 6h intervals UC (cumulative collection 12-18h, and 18-24h postdose).
- Urine samples for renal injury biomarkers and creatinine (from the last void of the 0-12h collection) (Section 7.5.1).
- 12-lead ECG recording at 3h, 6h, and 12h postdose taken after 5 minutes in a supine position.
- Assessment of local reaction of injection site(s) 45 min, 6h, and 12h postdose (Section 7.3.7).
- Blood sampling for retention at 3h postdose.
- AEs assessment and change in concomitant medications.
- Meals will be served.

The study staff will encourage the subjects to drink at least one glass (approximately 250 mL) of fluids every hour up to 10h after dosing.

Day 2

- Vital signs (after 5 minutes in a supine position), BP, HR, RR, and oral temperature 24h).
- 12-lead ECG (24h) taken after 5 minutes in a supine position.

- Assessment of local injection(s) reaction (24h).

Blood sample collection:

  - Safety lab tests (24h)
  - PK (24h and 36h)
  - Blood sampling for housekeeping proteins (24h).
- Urine collection :
  - Urinalysis (24h)
  - Urine PK, 12h intervals (cumulative 24h-36h and 36-48h collection)
  - Renal injury biomarkers and creatinine (24h and 36h urine void)
- AEs assessment and change in concomitant medications.

Day 3 (Discharge from CPU)

Before discharge (at approximately 48 hours after dosing), subjects will undergo the following assessments:

- Last urine collection added to the previous urine collection for PK.
- Urine samples for renal injury biomarkers, creatinine and general urinalysis (48h urine void).
- Assessment of local injection(s) reaction (48h).
- Vital signs (supine BP, HR, RR, and oral temperature) after 5 min in a supone position (48h)
- Weight.
- Physical examination (48h).
- 12-lead ECG (48h) taken after 5 minutes in a supine position
- Blood samples collection (48h):
  - Safety lab tests (48h)
  - PK (48h)
  - Blood sampling for housekeeping proteins (48h).
- AEs assessment and change in concomitant medication.
- Auditory and vestibular assessments (may be performed on Day 2, as well if not otherwise possible).

Subjects will be discharged following satisfactory clinical assessment by the Investigator. Two urine jars will be provided to the subject for 48-72h urine collection at home.

Day 4-Day 11-Day 18

Subjects will hand over the urine jars to the site personnel (only on Day 4), for the urine PK sampling from the collection 48-72h.

Predose

- Vital signs (supine BP, HR, RR, and oral temperature).
- Weight (if not taken at previous day).

- Urine samples for creatinine and renal injury biomarkers.
- AEs assessment and change in concomitant medication.
- Subjects will drink at least 500 mL water within 120 minutes before dosing.
- A PK blood sample will be taken 180 min before study drug administration.

Study drug administration

Each subject will receive ELX-02 or Placebo with SC injection, while confined to bed, as described in Section 5.4. Time and date of drug administration will be documented.

Postdose

- Local reaction of injection site (up to 1h postdose).
- PK blood sample (1h).

The study staff will encourage the subjects to drink at approximately 250 mL of fluids every hour up to 10h after dosing.

Subjects will remain at the CPU for 2h following study drug administration for safety observation.

Day 10-Day 17-Day 24

Auditory and vestibular assessments (may be performed on Day 9, Day 16, or Day 23, respectively, as well if not otherwise possible).

Weight (to be assessed in the morning)

Day 7-Day 14-Day 21

Subjects will come to the CPU for safety blood/urine sampling and AE/CM assessments, weight and stay confined till the last assessment the day after.

- UDS on Day 14
- Serum alcohol on Day 14
- Blood safety and general urinalysis.
- AEs assessment and change in concomitant medication.
- Weight (to be assessed in the morning).

Day 8-Day 15-Day 22

Predose

- Serum alcohol on Day 22
- Vital signs (supine BP, HR, RR, and oral temperature).
- Weight (if not taken at previous day).
- Physical examination.
- 12-lead ECG taken after 5 minutes in a supine position.

- Urine samples for creatinine and renal injury biomarkers.
- Blood sampling for housekeeping proteins.
- Urine pregnancy test (all women regardless of childbearing potential)
- Blood sampling for retention.
- AEs assessment and change in concomitant medication.
- Subjects will drink at least 500 mL water within 120 minutes before dosing.
- A PK blood will be taken right before dosing.

Study drug administration

Each subject will receive ELX-02 or Placebo with SC injection, while confined to bed. Date and time of drug administration will be documented.

Postdose

- Assessment of local injection(s) reaction (up to 1h postdose).
- PK blood sample (1h).

The study staff will encourage the subjects to drink at least one glass (approximately 250 mL) of fluids every hour up to 10h after dosing.

Subjects will remain at the CPU for 2h following study drug administration for safety observation.

Day 25

Predose

- Urine samples for creatinine and renal injury biomarkers.
- Vital signs (supine BP, HR, RR, and oral temperature).
- Weight.
- AEs assessment and change in concomitant medication.
- Subjects will drink at least 500 mL water within 120 minutes before dosing.
- A PK blood sample will be taken right before study drug administration.

Study drug administration

Each subject will receive ELX-02 or Placebo with SC injection, while confined to bed. Date and time of drug administration will be documented.

Postdose

The study staff will encourage the subjects to drink at least one glass (approximately 250 mL) of fluids every hour up to 10h after dosing.

- Assessment of local injection(s) reaction (up to 1h postdose).
- PK blood sample (1h postdose).

Day 28 (Check-in)

Subjects will be admitted to the CPU on Day 28 for safety blood and urine sampling and will be interviewed by study personnel regarding concomitant medications and any change in their health status or diet since the previous visit.

- Physical examination.
- Blood safety and general urinalysis.
- Weight

Subjects will stay confined till the morning of Day 31.

The following assessments will be done :

- Vital signs (supine BP, HR, RR, and oral temperature) taken after 5 minutes in a supine position.
- Urine pregnancy test (all women regardless of childbearing potential).
- Urine Drug Screen (UDS).
- Serum alcohol test.
- Urine samples for creatinine and renal injury biomarkers.
- Assessment of AEs and concomitant medications.

Day 29

Predose

Prior to drug administration, the following activities will be completed:

- First morning urine sample for PK determination, for creatinine, renal injury biomarkers assessment.
- Vital signs (supine BP, HR, RR, and oral temperature) taken after 5 minutes in a supine position.
- 12-lead ECG taken after 5 minutes in a supine position.
- Assessment of AEs and concomitant medications.
- Weight (if not already taken on Day 28).
- Subjects will drink at least 500 mL water within 120 minutes before dosing.
- Blood samples for PK, housekeeping proteins, and retention (a retention sample will be taken to assess any potential safety issues e.g hematology and biochemistry, coagulation) will be drawn. The PK blood sample will be taken right before study drug administration.

Study Drug administration

Each subject will receive ELX-02 or Placebo with SC injection, while confined to bed, as described in Section 5.4. Date and time of drug administration will be documented.

Postdose

- After dosing, subjects will remain confined to bed for four hours (except when required to void). They will then be allowed to move freely within the ward but must refrain from strenuous physical activity for the duration of the study.
- Blood samples for PK will be collected at 15 min, 30 min, 45 min, 1h, 3h, 6h, and 12h postdose (Section 7.4).
- Vital signs (supine BP, HR, RR, and oral temperature) will be measured at 30 min, 45 min, 1h, 3h, and 12h postdose.
- Brief physical examination at 6h postdose.
- Blood and urine safety test at 6h postdose.
- Hourly Urine collection for PK during 0-12h (each void collected separately) and 12-24h postdose (cumulative collection in 6h intervals ; 12-18h, and 18-24h postdose).
- Urine samples for renal injury biomarkers and creatinine (from the last void of the 0-12h collection) (Section 7.5.1).
- 12-lead ECG recording at 3h, 6h, and 12h postdose taken after 5 minutes in a supine position.
- Assessment of local reaction of injection site(s) 45 min, 6h, and 12h postdose (Section 7.3.7).
- Blood sampling for retention (at 3h postdose).
- AEs assessment and change in concomitant medications.
- Meals will be served.

The study staff will encourage the subjects to drink at least one glass (approximately 250 mL) of fluids every hour up to 10h after dosing.

Day 30

- Vital signs (supine BP, HR, RR and oral temperature, 24h).
- 12-lead ECG (24h) taken after 5 minutes in a supine position.
- Blood sample collection:
    - Safety lab tests (24h)
    - PK (24h and 36h)
    - Blood sampling for housekeeping proteins (24h).
- Urine collection:
    - Urinalysis (24h)
    - PK (12h intervals cumulative 24h-36 and 36-48h)
    - Renal injury biomarkers and creatinine (24h and 36h urine void)
- AEs assessment and change in concomitant medications.
- Local reaction of injection site (24h).

Day 31 (Discharge from CPU)

Before discharge (at approximately 48 hours after dosing), subjects will undergo the following assessments:

- Last urine collection added to the previous urine collection for PK.
- Urine samples for renal injury biomarkers, creatinine and general urinalysis. (48h urine void).
- Assessment of local injection(s) reaction (48h).
- Vital signs (supine BP, HR, RR, and oral temperature) (48h).
- Physical examination (48h).
- 12-lead ECG (48h) taken after 5 minutes in a supine position.
- Blood sampling for safety lab tests (48h).
- Blood sampling for PK (48h).
- Blood sampling for housekeeping proteins (48h).
- AEs assessment and change in concomitant medication.

Subjects will be discharged following satisfactory clinical assessment by the Investigator. Two urine jars will be provided to the subject for 48-72h urine collection at home.

Day 32

Subjects come to the CPU in the morning for a 72h PK blood sample. They will hand over the urine jars to the site personnel, for the urine PK sampling from the collection 48-72h. The site personnel will check if any AE and/or change in concomitant medications occurred.

End of Study Visit (Day 36)

This visit will take place 7-10 days after last dosing. Auditory and vestibular assessments may take place 7-11 days after last dosing.

The following assessments will take place:

- Physical examination.
- Assessment of local injection(s) reaction.
- Vital signs (supine BP, HR, RR, oral temperature).
- 12-lead ECG taken after 5 minutes in a supine position.
- Blood and urine safety tests.
- Serology (HIV, HBsAg, HCVAb).
- Urine pregnancy test (all women regardless of childbearing potential).
- Urine samples for creatinine and renal injury biomarkers.
- Serum alcohol test.
- Blood sampling for housekeeping proteins.
- AEs assessment and change in concomitant medications.
- Audiometry tests.
- Vestibular and tinnitus questionnaires.

Unscheduled Visit

An unscheduled visit may take place at any time during the study at the subject's request or as deemed necessary by the Investigator due to medical considerations. The date and reason for the unscheduled visit will be recorded. AE monitoring and concomitant medication recording will be recorded. Other procedures and evaluations will be completed as deemed necessary by the Investigator and may include (but not limited to) safety laboratory tests, ECG, vital signs and physical examination.

Handling Subject Withdrawal

If a subject's participation in the study is prematurely terminated for any reason or fails to return for a scheduled visit, every effort should be made to determine the reason. This information will be recorded.

Upon withdrawal from the study, any time after dosing has taken place, all efforts should be made to invite the subject to complete the EOS visit.

If for any reason the subject does not agree to return to the CPU for the above visit, the reason and/or efforts made will be recorded.

If the subject withdraws from the study and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The Sponsor may retain and continue to use any data collected before such withdrawal of consent.

7.2. Allowed Time Window Deviations

Allowed time window deviations are (assessments at screening, Day -1, and EOS not described here):

Vital signs

Day 1 and Day 29: predose, and 30 min (± 5 min), 45 min (± 5 min), 1h (± 15 min), 3h (± 15 min), 12h (± 30 min), 24h (± 1h), 48h (± 1h) postdose.

On other dosing days, vital signs need to be assessed before dosing.

Physical examination

Day 1 and Day 29: 6h (± 1h), 48h (± 2h) postdose.

On other dosing days, the physical examination needs to be performed before dosing.

ECG

Day 1 and Day 29: predose, and 3h (± 15 min), 6h (± 15 min), 12h (± 30 min), 24h (± 1h), 48h (± 1h) postdose.

On other dosing days, a 12-lead ECG needs to be taken before dosing.

Audiometric testing

The allowed time window deviations for audiometric and vestibular testing during the treatment period are Screening (-42 to -8 days), Baseline (-7 to Day -1) (these two tests can be performed on the same day) Day 3 (-1 day), Day 10 (-1 day), Day 17 (-1 day), Day 24 (-1 day), and EOS (7-11 days after last dosing). The otoscopic exam on Day 3, Day 10, Day 17 and Day 24 can be performed 72 hours prior to dosing.

Local reaction of injection

Day 1 and Day 29: 45 min (± 5 min), 6h (± 15 min), 12h (± 30 min), 24h (± 1h), 48h (± 1h) postdose.

On other dosing days the local reaction of injection needs to be assessed up to 1h post dosing.

For laboratory samples

a. Blood samples

- Safety sampling: Day 1 and Day 29: 6h (± 15 min), 24h (± 15 min), 48h (± 30 min). Other safety blood samples will be taken in the morning of Day 7, Day 14, Day 21, and Day 28.

- PK sampling: Day 1 and Day 29: predose, 15 min (± 2 min), 30 min (± 5 min), 45 min (± 5 min), 1h (± 5 min), 3h (± 15 min), 6h (± 15 min), 12h (± 30 min),

24h (± 1h), 36h (± 1h), 48h (± 1h), 72h (± 1h). On other dosing days, the PK blood samples will be taken predose and 1h postdose (± 5 min).

- Housekeeping proteins: Day 1 and Day 29: predose and 24h (± 1h), 48h (± 1h) postdose. On other dosing days the sample needs to be taken predose.

- Blood retention sampling: Day 1 and Day 29 predose and at 3h (± 15 min) postdose. On other dosing days the sample needs to be taken predose.

b. Urine samples

- Safety sampling: Day 1 and Day 29: 6h (± 30 min), 24h (± 2h), 48h (± 2h). Other safety urine samples will be taken in the morning of Day 7, Day 14, Day 21, and Day 28.

- PK sampling: Day 1 and Day 29: predose and during 0-12h (± 15 min), 12-24h (± 15 min), 24-48h (± 15 min), and 48-72h (± 15 min) postdose. And on Day 8, 11, 15, 18, 22, and 25 for creatinine.

- Renal injury biomarkers and creatinine sampling: predose and 12h sample from last void of the 0-12h collection (± 30 min), 24h (± 2h), 36h (± 2h), 48h (± 2h), and 72h (± 2h) postdose. And on Day 8, 11, 15, 18, 22, and 25.

7.3. Safety Evaluations

7.3.1. Adverse Events (AEs)

Adverse events will be collected continuously starting from signing the Informed Consent Form (ICF) until EOS visit for randomized subjects.

Adverse events reported prior to dosing will be considered non treatment emergent. Any new systemic effect that occurs between scheduled visits should be brought to the attention of the Investigator and recorded in the subject's files.

Common Terminology Criteria for Adverse Events (CTCAE[21]) will be used to determine the AE safety profile of ELX-02. For more information refer to Section 8.

7.3.2. Concomitant Medications

Use and any changes in concomitant medication will be recorded continuously starting from screening until EOS visit. Contraindicated drugs are listed in Section 6.1.

7.3.3. Vital Signs

Vital signs (supine BP, HR, RR, and oral temperature) will be measured in supine position after at least 5 min of supine rest at the time points specified in Section 7.1 and in Table 1.

On days when vital signs parameters need to be assessed as well as blood sampling, the vital signs will be performed before blood sampling, except when an indwelling cannula has been placed.

Changes in vital signs determined by the Investigator to be clinically significant will be noted as an AE in the eSource. Such abnormalities will be closely monitored until stabilized or resolved.

7.3.4. Physical Examination

Complete physical examination will be performed at the time-points specified in Section 7.1 and in Table 1.
Significant changes from baseline examination on screening will be recorded as AEs.

7.3.5. ECG Examination

A 12-lead ECG (taken after 5 minutes in a supine position) will be performed at the timepoints specified in Section 7.1 and in Table 1.

The following values will be collected: QT, QTc, HR, QRS, and PR.

On days when a 12-lead ECG parameters needs to be assessed as well as blood sampling, the vital signs and 12-lead ECG will be performed before blood sampling, except when an indwelling cannula has been placed.

Any ECG abnormality compared to baseline (Day 1 predose) reading determined by the Investigator to be clinically significant will be recorded as an AE. Such abnormalities will be closely monitored until stabilized or resolved.

7.3.6. Auditory and Vestibular Assessments

The following auditory and vestibular tests will take place:

- Otoscopy at all visits (the otoscopic exam on Day 3, Day 10, Day 17 and Day 24 can be performed 72 hours prior to dosing by a CPU physician)
- Tympanometry (Immittance audiometry) at all visits
- Speech Reception Threshold (SRT)- will be performed at screening and baseline. At follow up visits, SRT will be performed only if there are ASHA significant change criteria/reverse ASHA significant change criteria noted in the PTA/HFA exam.
- Pure tone audiometry (PTA) with frequencies up to 8 kHz if possible. Should there be an air conduction threshold of ≥15 dB, the subject should undergo bone conduction testing at that frequency if that threshold is for the frequency is 4 kHz or lower.
- High frequency audiometry (HFA) up to 16 kHz if possible.
- Tinnitus questionnaires (Tinnitus Handicap Inventory)
- Vestibular questionnaires (Dizziness Handicap)

For all tests, the same equipment should be used, as well as any repeat test to minimize technical variability.

More details can be found in Section 8.4.2. For description of these assessments refer to the Auditory and Vestibular Tests Manual.

All audiometric findings will be written on an auditory entry form and abnormal findings will be sent to the AVDSMB (consisting of a physician and audiologist) when required. The AVDSMB will decide if further dosing will occur, taking into account the stopping criteria.

7.3.7. Local Reaction of Injection Site

The extent of local reaction on the injection site will be graded according to CTCAE 4.03, modified from the Food and Drug Administration (FDA) Guidance for Industry (FDA, 2007[22]) and DAIDS criteria (Section 13.1).
Local reaction will be assessed at the timepoints specified in Section 7.1 and in Table 1.

7.3.8. Pregnancy Testing

For female subjects of childbearing potential, a serum or urine pregnancy test (all women regardless of childbearing potential) will be performed at the timepoints specified in Section 7.1 and in Table 1.
A negative pregnancy result is required before the subject may receive the investigational product. Pregnancy tests will also be done whenever 1 menstrual cycle is missed during the active treatment period (or when potential pregnancy is otherwise suspected), and at the end of the study to confirm the subject has not become pregnant during the study. Pregnancy tests may also be repeated as per request of institutional review boards (IRBs)/Independent ethics committees (IECs) or if required by local regulations.

In the case of a positive confirmed pregnancy, the subject will be withdrawn from administration of investigational product.

If a study subject or study subject's partner becomes or is found to be pregnant during the subject's treatment with ELX-02, the Investigator must submit this information on a pregnancy report form to the CRO within 24 h of their knowledge of the event, preferably by fax or by e-mail. Contact information will be provided in a separate document.

If a participating s ubject's partner becomes pregnant during the study period, the Investigator will request her to sign an informed consent before following up her pregnancy course.

Follow-up is conducted to obtain general information on the pregnancy and its outcome. The Investigator will follow the pregnancy until completion (or until pregnancy termination) and notify the Sponsor of the outcome on a follow-up report.

If the outcome of the pregnancy meets the criteria for an SAE (i.e. ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly [in a live-born baby, a terminated fetus, an intrauterine fetal demise, or a neonatal death]), the Investigator should follow the procedures for reporting SAEs.

More information can be found in Section 8.14.

7.3.9. Safety Blood and Urine, and Blood Sampling for Retention

Safety laboratory evaluations and blood sampling for retention will be performed at the timepoints specified in Section 7.1 and in Table 1.

All blood samples for retention need to be taken after an overnight fast of at least 8h, except for the samples taken at 3h postdose on Day 1 and Day 29. Processing and storage of the samples are detailed in the laboratory manual provided by the Sponsor.

All blood samples for safety assessments should be taken after an overnight fast of at least 8h, except for the samples taken at 6h postdose on Day 1 and Day 29. At screening subjects will be instructed not to eat within 3h before arrival at the CPU.
The following safety laboratory assessment variables will be analyzed:

Table 2: Safety Test

Safety Blood Tests			
Hematology and Coagulation			
Red Blood Cell (RBC) count	Mean corpuscular hemoglobin (MCH)	White blood cells (WBC	International normalized ratio (INR)
Hemoglobin	Mean corpuscular hemoglobin concentration (MCHC)	Platelets	WBC differential count
Hematocrit	Mean cell volume (MCV)	Prothrombin	
Biochemistry			
Total Protein	Aspartate aminotransferase (AST)	Alkaline Phosphastase (ALP)	Blood urea nitrogen
Albumin	Gamma glutamyl transferase (GGT)	Glucose	Serum Creatinine
Total Bilirubin	Lactate dehydrogenase (LDH)	Natrium (Sodium)	Glomerular filtration rate (GFR), (MDRD) (only at screening visit)
Alanine aminotransferase (ALT)	Creatine Phosphokinase	Potassium	
Hormones			
Serum β-human chorionic gonadotropin (HCG) (screening only)		FSH (screening only)	
Serology (screening and follow-up only)			
HIV Ab	HBsAg	HCV Ab	
Urine Tests			
Urinalysis			
Proteins	Ketones	pH	Specific Gravity (SG)
Glucose	Urobilinogen	RBC	Bilirubin
Urine protein and Albumin (screening and EOS)			

Urine Biochemistry			
Creatinine			
Hormones			
Urine pregnancy test (all women, regardless of childbearing potential) Day -1, Day 7, Day 14, Day 21, Day 28, Day 36			
Drugs of Abuse (screening) Day -1, Day 7, Day 14, Day 21, and Day 28.			
Cannabinoids (THC)	Amphetamines	Benzodiazepines	Opiates
Serum alcohol			

Abnormal safety tests may be confirmed by a single repeat, if deemed necessary.

The following microscopic parameters will be tested: RBC, WBC, bacteria, epithelial cells, granular casts, and hyaline casts.

After dosing, all lab tests outside the normal range will be repeated as clinically indicated until the values return to normal, or until the etiology has been determined and the condition considered stable. Abnormal laboratory test results that are considered to be clinically significant by the Investigator will be reported as an AE.

7.3.9.1. Total Volume of Blood Sampling

The total volume of blood that will be drawn from each subject will be approximately 542 mL for male and 547 mL for female subjects over a period of 12 weeks.

If necessary, in order to obtain additional information to ensure a subject's safety, additional blood samples (up to 50 mL) and/or urine samples may be taken at the discretion of the Investigator.

7.4. ELX-02 Pharmacokinetic Assessments

7.4.1. Blood Sampling

Blood samples for determination of ELX-02 plasma concentrations will be taken using tubes containing K3EDTA at the timepoints specified in Section 7.1 and in Table 1.
Before blood draw, the sample will be identified with a coded label, bearing the following details:

- Study Number
- Subject Number
- Time Point

Sample handling, processing and shipment are described in the laboratory manual.

The actual sample collection date and exact clock time will be recorded. Sampling problems will be noted in the eSource.

Blood may be drawn either by direct venipuncture or through an indwelling IV cannula. If meals and blood collections coincide, blood will be collected before eating.

For any additional information related to laboratory related procedures, requirements and supplies, refer to the Laboratory Manual.

7.4.2. Urine Collection

Urine samples, to measure the rate of ELX-02 excretion, will be collected at the timepoints specified in Section 7.1 and in Table 1.

- Day 1: Predose collection (first void in the morning). Urine samples for PK, biomarker, and creatinine analysis will be collected. Specific gravity will be calculated for the collection.
- Day 1 (0-12 hours postdose collection period): Subjects will be encouraged to void as frequently as possible (i.e., asked every hour if they can collect urine), and each individual sample will be collected separately. Each sample's weight will be recorded individually prior to removing urine for analyses. Specific gravity will be calculated for each collection.
- Day 1: 12-24 hours postdose collection period. This urine collection period includes all voids starting after 12h and includes the first void on the next day (i.e., 24h postdose void).
  The urine will be collected every 6h (i.e., 12-18h collection and 18-24h collection). The 12-18h voids and the 18-24h voids will be collected into separate urine collection containers.
  In addition, a single urine collection will be taken 24 hours after dosing using a third container (i.e., first morning void of Day 2). All urine containers will be weighed individually and the weights will be recorded. Urine samples for urinalysis, biomarker and creatinine analysis will be withdrawn from the single 24h urine void. Urine samples for PK analysis will be withdrawn from the pooled 12-18h and pooled 18-24h urine collection containers. Specific gravity will be calculated for each collection.
- Day 2 – Day 3: 24-48 hours postdose collection period. This urine collection period includes all voids subsequent to the 24h postdose void and includes the first void on the next day (i.e., 48 hours postdose void).
  The urine will be collected every 12h (i.e. 24-36h collection and 36-48h collection). The 24-36h voids and the 36-48h voids will be collected into separate urine collection containers.
  In addition, a single urine collection will be taken 36 hours after dosing using a third container and a single urine collection will be taken 48 hours after dosing using a fourth container (i.e. the first morning void of Day 3). All urine containers will be weighed individually and the weights will be recorded. Urine samples for urinalysis will be withdrawn from the single 48h urine void. Urine samples for biomarker and creatinine analysis will be withdrawn from the single 36h and 48h urine void. Urine samples for PK analysis will be withdrawn from the pooled 24-36h and pooled 36-48h urine collection containers. Specific gravity will be calculated for each collection.
- Day 3 – Day 4: 48-72 hours postdose collection period. This urine collection period includes all voids subsequent to the 48h postdose void and includes the first void on the next day (i.e., 72 hours postdose void).

During this collection period, subjects will be at home and collect the urine in urine jars (provided by the study site personnel). During this period, voids will be collected into a urine collection container. In addition, a single urine collection will be taken 72 hours after dosing using a second container. Urine samples for biomarker analysis and creatinine will be withdrawn from the single 72h urine void. Urine samples for PK analysis will be withdrawn from the pooled 48-72h urine collection container. Gravity will be calculated for each collection.

- Day 29: Predose collection (first void in the morning). Urine samples for PK, biomarker, and creatinine analysis will be collected. Specific gravity will be calculated for the collection.
- Day 29 (0-12 hours postdose collection period): Subjects will be encouraged to void as frequently as possible (i.e., asked every hour if they can collect urine), and each individual sample will be collected separately. Each sample's weight will be recorded individually prior to removing urine for analyses. Specific gravity will be calculated for each collection.
- Day 29: 12-24 hours postdose collection period. This urine collection period includes all voids starting after 12h and includes the first void on the next day (i.e., 24h postdose void).
  The urine will be collected every 6h (i.e., 12-18h collection and 18-24h collection). The 12-18h voids and the 18-24h voids will be collected into separate urine collection containers.
  In addition, a single urine collection will be taken 24 hours after dosing using a third container (i.e., first morning void of Day 30). All urine containers will be weighed individually and the weights will be recorded. Urine samples for urinalysis, biomarker and creatinine analysis will be withdrawn from the single 24h urine void. Urine samples for PK analysis will be withdrawn from the pooled 12-18h and pooled 18-24h urine collection containers. Specific gravity will be calculated for each collection.
- Day 30 – Day 31: 24-48 hours postdose collection period. This urine collection period includes all voids subsequent to the 24h postdose void and includes the first void on the next day (i.e., 48 hours postdose void).
  The urine will be collected every 12h (i.e. 24-36h collection and 36-48h collection). The 24-36h voids and the 36-48h voids will be collected into separate urine collection containers.
  In addition, a single urine collection will be taken 36 hours after dosing using a third container and a single urine collection will be taken 48 hours after dosing using a fourth container (i.e. the first morning void of Day 31). All urine containers will be weighed individually and the weights will be recorded. Urine samples for urinalysis will be withdrawn from the single 48h urine void. Urine samples for biomarker analysis and creatinine will be withdrawn from the single 36h and 48h urine void. Urine samples for PK analysis will be withdrawn from the pooled 24-36h and pooled 36-48h urine collection containers. Specific gravity will be calculated for each collection.
- Day 31 – Day 32: 48-72 hours postdose collection period. This urine collection period includes all voids subsequent to the 48h postdose void and includes the first void on the next day (i.e., 72 hours postdose void).

During this collection period, subjects will be at home and collect the urine in urine jars (provided by the study site personnel). During this period, voids will be collected into a urine collection container. In addition, a single urine collection will be taken 72 hours after dosing using a second container. Urine samples for biomarker analysis and creatinine will be withdrawn from the single 72h urine void. Urine samples for PK analysis will be withdrawn from the pooled 48-72h urine collection container. Specific gravity will be calculated for each collection.

After weighing and removing urine for analyses, the collection containers with remaining urine may be discarded.

If urine cannot be obtained at a certain time interval it will be duly recorded and urine will be collected at the next time interval.

Urine collection will be timed and measured for weight to determine the retained fraction of ELX-02 concentration and delay of excretion of the retained fraction and the PK.

7.4.3. Bioanalysis

Samples for determination of the concentration of ELX-02 will be analyzed by a qualified vendor (Aptuit) under the responsibility of the Sponsor, using a validated analytical method (HPLC-MS/MS). Instructions for laboratory processing will be detailed in the laboratory manual.

The laboratory analysis will be carried out following the principles of Good Laboratory Practice (GLP) regulations of the Organization for Economic Co-operation and Development (OECD). The analytical procedure will be described in a separate analytical protocol. Validation data and details of the analytical procedure will be gathered in an analytical report that will be attached to or referred to in the Clinical Study Report upon completion of the study.

Samples that remain after protocol-specific assessments have been performed may be used for further exploratory work on pharmacokinetics, metabolites, plasma protein binding, protein analysis, and biochemistry. No human DNA or RNA analysis will be performed.

7.4.4. Pharmacokinetic Parameters

The following parameters, where appropriate, will be determined for ELX-02, according to the definitions and methods of calculation below:

- C_{max} and t_{max} Maximum plasma concentration and corresponding time, directly obtained from the experimental data of plasma concentration vs time curves, without interpolation;
- AUC_t Area under the plasma concentration-time curve calculated from time of administration to the last quantifiable concentration, computed using the linear trapezoidal rule;
- AUC_{24h} Area under the plasma concentration-time curve calculated from time of administration to time 24h, computed using the linear trapezoidal rule;
- AUC_{48h} Area under the plasma concentration-time curve calculated from time of administration to time 48h (after first dose only), computed using the linear trapezoidal rule;

	AUC_{72h}	Area under the plasma concentration-time curve calculated from time of administration to time 72h (after first dose only), computed using the linear trapezoidal rule;
	AUC_{inf}	The area under the plasma concentration-time curve extrapolated to infinity, calculated as AUC_t + Clast / λz, where Clast is the last concentration above the lower limit of quantification (LLOQ;
-	$C_{predose}$	Trough plasma concentration observed at the end of the dosing interval (i.e. at each predose starting from second dose);
-	C_{1h}	Plasma concentration observed at 1h postdose at each dosing starting from second dose;
-	$t_{1/2}$	Apparent elimination half-life associated with the terminal rate constant (λ_z), calculated as $\ln 2/\lambda_z$;
-	Vd/F	Apparent volume of distribution;
-	CL/F	Apparent plasma clearance;
-	Rac	Accumulation ratio, calculated as AUC_{72h} Day29/AUC_{72h} Day 1.

Additional pharmacokinetic parameters may be calculated as appropriate.

The following parameters will be calculated based on ELX-02 urine concentrations: urinary excretion (Ae) of ELX-02 (in mass and %dose - fe) for each collection interval, cumulated urinary excretion (in %dose) for each collection interval, total excretion (in %dose) and renal clearance (using the AUC and Ae for the same time interval).

7.5. Pharmacodynamics

7.5.1. Renal Injury Biomarkers

Urine samples (2 mL) for early markers of renal injury (KIM-1 and clusterin) will be collected at the timepoints specified in Section 7.1 and in Table 1.
Processing and storage of the samples are detailed in the lab manual provided by the Sponsor.

7.5.2. Potential Read-Through of Housekeeping Protein

Blood samples (2 x 8 mL) for determination of ELX-02 effect on housekeeping proteins will be taken at the timepoints specified in Section 7.1 and in Table 1.

Processing and storage of the samples are detailed in the laboratory manual provided by the Sponsor.

The tubes will be pre-identified with details as follows:

- Study Number
- Time Point
- Sample type (peripheral blood mononuclear cells; PBMC)

The study staff will complete the following details:

- Randomization Number
- Actual date and time of sampling

Every effort should be made to ensure that the protocol-required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances, outside of the control of the Investigator that may make it unfeasible to perform the test. In these cases, the Investigator will take all steps necessary to ensure the safety and well-being of the subject. When a protocol-required test cannot be performed, the Investigator will document the reason for this and any corrective and preventive actions that he or she has taken to ensure that normal processes are adhered to as soon as possible. The study team will be informed of these incidents in a timely fashion.

7.6. Mitochondrial Mutations Testing

Blood samples for determination of mitochondrial mutations testing will be taken using tubes containing K3EDTA at the timepoints specified in Section 7.1 and in Table 1. One back-up sample will be taken. The samples will be shipped ambient within 5 days to the Cidegen laboratory in Spain.

Further details will be specified in a laboratory manual provided by the Sponsor.

8. ADVERSE EVENT REPORTING

8.1. Adverse Events

All observed or volunteered AEs regardless of treatment group suspected causal relationship to the investigational product(s) will be reported as described in the following sections.

For all AEs, the Investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets the criteria for classification as a SAE requiring immediate notification to Eloxx Pharmaceuticals or its designated representative. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE. The Investigator is required to assess causality. 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.

To assist in the determination of case seriousness, further information may be requested from the Investigator to provide clarity and understanding of the event in the context of the clinical study.

8.2. Reporting Period

For SAEs, the active reporting period to Eloxx Pharmaceuticals or its designated representative begins from the time that the subject provides informed consent, which is obtained prior to the subject's participation in the study, ie, prior to undergoing any study-related procedure and/or receiving investigational product, through and including 28 calendar days after the last administration of the investigational product. SAEs occurring to a subject after the active reporting period has ended should be reported to the sponsor if the Investigator becomes aware of them; at a minimum, all SAEs that the Investigator believes have at least a reasonable possibility of being related to investigational product are to be reported to the sponsor.

8.3. Definition of an Adverse Event

An AE is any untoward medical occurrence in a clinical investigation subject administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage. Examples of AEs include but are not limited to:

- Abnormal test findings;
- Clinically significant symptoms and signs;
- Changes in physical examination findings;
- Hypersensitivity;
- Drug abuse;
- Drug dependency.

Additionally, they may include the signs or symptoms resulting from:

- Drug overdose;
- Drug withdrawal;
- Drug misuse;
- Drug interactions;
- Extravasation;
- Exposure during pregnancy (EDP);
- Exposure via breastfeeding;
- Medication error;
- Occupational exposure.

8.4. Adverse Events of Interest

For this study, Adverse Events of Interest (AEOI) include the following:

a. Hypersensitivity – graded based on CTCAE[21].
b. Nephrotoxicity – graded based on CTCAE[21]. Further criteria are described in Section 8.4.1.
c. Ototoxicity – graded based on CTCAE[21]. Further criteria are described in Section 8.4.2. Note: For all adverse events of interest related to Ear and Labyrinth disorders, the adjudication committee will assess the grading.

Adverse events of interest need to be reported immediatelyto the Sponsor Medical Respresentative. The Sponsor will report adverse events of interest to the DSMB and AEOIs related to ototoxicity will be reported to the AVDSMB.

An AEOI report must be completed and sent via fax or email within 24 hours of Investigator's knowledge of the event to the study monitor and Sponsor's representative.

In case the AEOI is classified as SAE, only the SAE report will be completed and sent to the CRO, preferably by fax or by e-mail. Contact information will be provided in a separate document. More details are provided in Section 8.21.2.

8.4.1. Criteria for Nephrotoxicity

Urine samples (2 mL) for early markers of renal injury (KIM-1 and clusterin) will be used for as early indicator. Creatinine will be evaluated for safety determination at specified time-points prior to and after study drug administration to detect potential renal injury.

Grading of any nephrotoxic event shall be based on CTCAE[21].

8.4.2. Criteria for Ototoxicity

Grading of ototoxicity will be done by the adjudication committee appointed to the study in accordance with the CTCAE criteria.

Additional measures will be described in the Auditory and Vestibular Tests Manual.

1. Tympanometry

Abnormalities in tympanometry alone will not determine ototoxicity, but will be used to ascertain normal middle ear compliance and the presence of a conductive hearing loss that may make interpretation of testing impossible.

2. Speech Reception Threshold:

Speech reception threshold (SRT) will be used to determine the reliability of PTA measurements.

3. Pure Tone Audiometry:

Pure Tone Audiometry will be conducted across the conventional range of frequencies (250-8,000 Hz), as well as at high frequencies up to 16,000 Hz. Abnormalities consist of decreased responses in consecutive frequencies according to the CTCAE criteria. If there is a PTA threshold ≥15 dB for frequencies between 250-4000 Hz, bone conduction will be performed at that frequency. (Note: 15dB is within normal limits; bone conduction can only be performed at that frequency.)

4. High Frequency Audiometry

For high frequencies, a potentially ototoxic event is defined as audiometry testing resulting in a threshold shift of >25 dB averaged at 3 contiguous test frequencies in at least one ear. If ASHA significant change criteria are met (see below) high frequency audiometry should be repeated within 24 hours to confirm the results and the investigator should consult on ASHA significant change criteria with Eloxx Pharmaceuticals.

5. Tinnitus questionnaire (Tinnitus Handicap Inventory).

If a subject has a score of a 5 point increase in THI score from baseline accompanied by a shift in Grade (eg from Grade 1 to Grade 2), the event will be discussed between the Investigator, the medically-qualified sponsor representative and the medically-qualified sponsor representative and the adjudication committee to determine if further auditory testing will be required. Any findings consistent with drug-induced toxicity leading to tinnitus on formal evaluation will be re-evaluated 3 months after initial exam and, if persistent, will be deemed permanent.

6. Vestibular questionnaire (Dizziness Handicap).

If a subject has a score of 31 or greater on the DHI accompanied by a 5 point increase from baseline and an associated shift in category (eg from mild to moderate handicap), the event will be discussed between the Investigator, the medically-qualified sponsor representative and the adjudication committee to determine if further vestibular testing will be required. Any findings consistent with drug-induced vestibular toxicity on formal evaluation will be re-evaluated 3 months after initial exam and, if persistent, will be deemed permanent.

For both the PTA and HFA:

The Significant Change Criteria as defined by American Speech-Language-Hearing Association (ASHA) and American Academy of Audiology (AAA) will be used to trigger a review when the thresholds are worse by the following criteria:

- ≥20 dB change at any frequency,
- ≥10 dB change at any 2 adjacent frequencies, or
- loss of response at 3 consecutive frequencies where responses were obtained at baseline.

To be considered significant as an early warning for possible ototoxicity, changes showing a worsening meeting the above criteria must replicate 24 hours later with no indication of middle ear abnormality. Subjects serve as their own controls for ototoxic change, which is computed relative to baseline measures.

Abnormal tests should be repeated within 24 hours, preferably immediately (with the exception of the questionnaires). For all tests, the same equipment should be used for the screening and EOS, as well as any repeat test to minimize technical variability.If any of the tests meet the significant change criteria described above, this finding must be considered an ototoxic AE ("Adverse Event of Interest"), which must be reported immediately to the the Medical Monitor and the Sponsor. The Sponsor will update the DSMB and AVDSMB.

We define reverse ASHA significant change criteria as an improvement, rather than a worsening by the same amount of change. Reverse ASHA significant change criteria will be used only as a measure of variability or possible learning effect as the subject becomes more experienced in the test procedures.

All audiometric findings will be written on an auditory entry form and abnormal findings will be sent to the AVDSMB (consisting of a physician and audiologist) for review prior to each dosing (more details can be found in study administrative structure). The AVDSMB will decide if further dosing will occur, taking into account the stopping criteria (refer to Section 3.2).

Grading of any ototoxic event shall be based on CTCAE[21]. Further details are provided in the Auditory and Vestibular Tests Manual.

8.5. Medication Errors

Medication errors may result, in this study, from the administration or consumption of the wrong product, by the wrong subject, at the wrong time, or at the wrong dosage strength. Such medication errors occurring to a study participant are to be captured. In the event of medication dosing error, the sponsor should be notified immediately.

Medication errors are reportable irrespective of the presence of an associated AE/SAE, including:

- Medication errors involving subject exposure to the investigational product;
- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating subject.

Whether or not the medication error is accompanied by an AE, as determined by the Investigator, the medication error is captured on the medication error version of the AE page and, if applicable, any associated AE(s) are captured on an AE CRF page.

8.6. Abnormal Test Findings

The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

- Test result is associated with accompanying symptoms; and/or
- Test result requires additional diagnostic testing or medical/surgical intervention; and/or
- Test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment, or other therapy; and/or
- Test result is considered to be an AE by the Investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

8.7. Serious Adverse Events

A SAE is any untoward medical occurrence at any dose that:

- Results in death;
- Is life-threatening (immediate risk of death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;

- Results in persistent or significant disability/incapacity (substantial disruption of the ability to conduct normal life functions);

- Results in congenital anomaly/birth defect.

- Medical and scientific judgment is exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject or may require intervention to prevent one of the other AE outcomes, the important medical event should be reported as serious. 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 hospitalization; or development of drug dependency or drug abuse.

A serious injury that can cause a serious deterioration in state of health can include:

- a life-threatening illness, even if temporary in nature;

- a permanent impairment of a body function or permanent damage to a body structure;

- a condition necessitating medical or surgical intervention to prevent the above 2 bulleted items

Examples: clinically relevant increase in the duration of a surgical procedure; a condition that requires hospitalization or significant prolongation of existing hospitalization;

- any indirect harm as a consequence of an incorrect diagnostic or in vitro diagnostic device test results when used within the manufacturer's instructions for use;

- fetal distress, fetal death, or any congenital abnormality or birth defects.

8.8. Hospitalization

Hospitalization is defined as any initial admission (even less than 24 hours) in a hospital or equivalent healthcare facility or any prolongation of an existing admission. Admission also includes transfer within the hospital to an acute/intensive care unit (eg, from the psychiatric wing to a medical floor, medical floor to a coronary care unit, or neurological floor to a tuberculosis unit). An emergency room visit does not necessarily constitute a hospitalization; however, the event leading to the emergency room visit should be assessed for medical importance.

Hospitalization does not include the following:

- Rehabilitation facilities;

- Hospice facilities;

- Respite care (eg, caregiver relief);
- Skilled nursing facilities;
- Nursing homes;
- Same-day surgeries (as outpatient/same-day/ambulatory procedures).

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical AE is not in itself an SAE. Examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new AE or with a worsening of the preexisting condition (eg, for workup of persistent pretreatment laboratory abnormality);
- Social admission (eg, subject has no place to sleep);
- Administrative admission (eg, for yearly physical examination);
- Protocol-specified admission during a study (eg, for a procedure required by the study protocol);
- Optional admission not associated with a precipitating clinical AE (eg, for elective cosmetic surgery);
- Hospitalization for observation without a medical AE;
- Preplanned treatments or surgical procedures. These should be noted in the baseline documentation for the entire protocol and/or for the individual subject;

Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as the AE, and the resulting appendectomy should be recorded as treatment of the AE.

8.9. Severity Assessment

Severe assessment will be performed per categories specified in the CTCAE guidelines[21].

8.10. Causality Assessment

The Investigator's assessment of causality must be provided for all AEs (serious and non-serious); the Investigator must record the causal relationship in the CRF, as appropriate, and report such an assessment in accordance with the SAE reporting requirements if applicable. An Investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an AE; generally the facts (evidence) or arguments to suggest a causal relationship should be provided. If the Investigator does not know whether or not the investigational product caused the event,

then the event will be handled as “related to investigational product” for reporting purposes, as defined by the sponsor. If the Investigator's causality assessment is "unknown but not related to investigational product," this should be clearly documented on study records.

In addition, if the Investigator determines that a SAE is associated with study procedures, the Investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with the SAE reporting requirements, if applicable.

Causality of each AE must be assessed according to the WHO-Uppsala Monitoring Center (UMC) system for standardized case causality assessment:

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

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.

8.11. Action Taken Regarding the Study Drugs

The action taken towards the study drugs must be described as follows:

- Permanently discontinued;
- Stopped temporarily;
- Dose reduced;
- Dose increased;
- No action taken;
- Unknown/Not applicable.

8.12. Outcome

The outcome of each AE must be rated as follows:

Outcome	Clarification
Recovered/Resolved	Subject has fully recovered with no residual effects observable
Recovered with sequelae/Resolved with sequelae	Subject has recovered with residual effects observable
Not yet recovered/Not Resolved	Subject status improved but has not yet been recovered
Ongoing/Not Recovered/Not Resolved	Subject has not recovered and has no improvement
Fatal	Resulted in death of the subject
Unknown	e.g. lost to follow

8.13. Unexpected Adverse Event

An unexpected AE is any AE which is not listed in the Investigator brochure or is not listed at the specificity or severity that has been observed for an unapproved test drug or package insert/summary of product characteristics for an approved product (package inserts are available separately at the participating center).

Serious Unexpected Suspected Adverse Reaction (SUSAR) is a serious adverse reaction assessed as unexpected by the Sponsor and that is judged by either the reporting Investigator or the Sponsor to have a reasonable causal relationship to a test drug.

8.14. Exposure During Pregnancy

For both unapproved/unlicensed products and for marketed products, an exposure during pregnancy occurs if:

1. A female becomes, or is found to be, pregnant either while receiving or having been exposed (eg, because of treatment or environmental exposure) to the investigational product; or the female becomes or is found to be pregnant after discontinuing and/or being exposed to the investigational product;

2. An example of environmental exposure would be a case involving direct contact with a Eloxx Pharmaceuticals product in a pregnant woman (eg, a nurse reports that she is pregnant and has been exposed to chemotherapeutic products).

3. A male has been exposed (eg, because of treatment or environmental exposure) to the investigational product prior to or around the time of conception and/or is exposed during his partner's pregnancy.

If a study subject or study subject's partner becomes or is found to be pregnant during the study subject's treatment with the investigational product, the Investigator must submit this information to the CRO on a pregnancy form. In addition, the Investigator must submit information regarding environmental exposure product in a pregnant woman (eg, a subject reports that she is pregnant and has been exposed to a cytotoxic product by inhalation or spillage). This must be done irrespective of whether an AE has occurred and within 24 hours of awareness of the exposure. The information submitted should include the anticipated date of delivery (see below for information related to termination of pregnancy).

Follow-up is conducted to obtain general information on the pregnancy and its outcome. The Investigator will follow the pregnancy until completion (or until pregnancy termination) and notify the CRO and Eloxx Pharmaceuticals of the outcome as a follow-up by forwarding the completed pregnancy form B. In the case of a live birth, the structural integrity of the neonate can be assessed at the time of birth. In the event of a termination, the reason(s) for termination should be specified and, if clinically possible, the structural integrity of the terminated fetus should be assessed by gross visual inspection (unless preprocedure test findings are conclusive for a congenital anomaly and the findings are reported).

Follow-up is conducted to obtain general information on the pregnancy and its. The Investigator will follow the pregnancy until completion (or until pregnancy termination) and notify the CRO and Eloxx Pharmaceuticals of the outcome as a follow-up by forwarding the completed pregnancy form B. In the case of a live birth, the structural integrity of the neonate can be assessed at the time of birth. In the event of a termination, the reason(s) for termination should be specified and, if clinically possible, the structural integrity of the terminated fetus should be assessed by gross visual inspection (unless preprocedure test findings are conclusive for a congenital anomaly and the findings are reported).

If the outcome of the pregnancy meets the criteria for an SAE (ie, ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly [in a live-born baby, a terminated fetus, an intrauterine fetal demise, or a neonatal death]), the Investigator should follow the procedures for reporting SAEs.

Additional information about pregnancy outcomes that are reported as SAEs follows:

- Spontaneous abortion includes miscarriage and missed abortion;
- Neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as SAEs. In addition, infant deaths after 1 month should be reported as SAEs when the Investigator assesses the infant death as related or possibly related to exposure to the investigational product.

Additional information regarding the exposure during pregnancy (EDP) may be requested by the Investigator. Further follow-up of birth outcomes will be handled on a case-by-case basis

(eg, follow-up on preterm infants to identify developmental delays). In the case of paternal exposure, the Investigator will provide the study subject with the Pregnant Partner Release of Information Form to deliver to his partner. The Investigator must document in the source documents that the subject was given the Pregnant Partner Release of Information Form to provide to his partner.

8.15. Follow-up of Adverse Events

Subjects who have had an AE during the treatment period must be followed clinically until all parameters (including laboratory) have either returned to normal or have stabilized or are otherwise explained.

Any newly emergent SAE within 30 days after early discontinuation or study completion, that is considered to be related to the test drug or study participation should be recorded and reported immediately. The post-study period for the purpose of SAE reporting is until the SAE is resolved or stabilized or maximum up to 30 days following last visit of the study.

Unless decided otherwise, the study will terminate 30 days after last subject has attended the End-of Study visit. If additional follow-up of an AE is warranted, it will be documented in the as the subject' source data but will not be part of the CRF. The PI is required to report on any significant findings to the Sponsor.

8.16. Independent Data and Safety Monitoring Board (DSMB)

A DSMB for the study will be formed to review the safety information generated for each cohort' subjects upon their completion of all EOS assessments.

The DSMB will be composed of four independent physicians specialized in relevant medical fields who will monitor general events. In addition, a separate Auditory and Vestibular DSMB composed of two ENT specialists will monitor and assess events which may be ototoxic in nature. The Sponsor's medically qualified representative and the Investigator and/or designee may attend the DSMB's open sessions but are not allowed to vote.

The primary responsibility of the DSMB is to evaluate the safety data collected for all subjects in the recently completed cohort and the safe conduct of the study and provides the Sponsor with a report summarizing the conclusions of the meeting, and its recommendation whether to proceed to the next cohort or otherwise. The decision to proceed to a higher dose level will be made by the Sponsor after considering the DSMB's, the advice of the AVDSMB, and the PI’s recommendation following the safety assessments and based on the dose-escalation stopping rules (Section 3.2).

Dose escalation will be permitted if the prior dose was well tolerated, if there were no safety or tolerability concerns. The next cohort will be dosed at least 14 days after the last dosing in the previous group. This window between cohort’s dosing can be extended in case the DSMB request for additional information to support its conclusion regarding the previous dose safety profile.

DSMB working procedures will be described in a DSMB charter prior to enrolling the first subject. The DSMB will prepare a report of its recommendations and will forward it to the Sponsor. The Sponsor will forward such decisions, which may include summaries of aggregate analyses of endpoint events and of safety data that are not endpoints, to regulatory authorities, as appropriate.

8.17. Occupational Exposure

An occupational exposure occurs when, during the performance of job duties, a person (whether a healthcare professional or otherwise) gets in unplanned direct contact with the product, which may or may not lead to the occurrence of an AE.

An occupational exposure is reported to the drug safety unit within 24 hours of the Investigator's awareness, using the SAE report form, regardless of whether there is an associated AE/SAE. Since the information does not pertain to a subject enrolled in the study, the information is not reported on a CRF; however, a copy of the completed SAE report form is maintained in the Investigator site file.

8.18. Withdrawal Due to Adverse Events (See Also the Section on Subject Withdrawal)

Withdrawal due to AEs should be distinguished from withdrawal due to other causes, according to the definition of AE noted earlier, and recorded on the appropriate AE CRF page.

When a subject withdraws because of an SAE, the SAE must be reported in accordance with the reporting requirements defined below.

8.19. Eliciting Adverse Event Information

The Investigator is to report all directly observed AEs and all AEs spontaneously reported by the study subject. In addition, each study subject will be questioned about AEs.

8.20. Recording of Adverse Events

All (S)AEs occurring during the clinical investigation must be documented in the eSource System.

Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record their opinion concerning the relationship of the (S)AE to the study drugs in the source documents and in the eSource System]. All measures required for (S)AE management must be recorded in the source documents and reported according to Sponsor's instructions.

All AEs occurring at any time during the study (including the follow-up period) will be followed by the Investigator until satisfactory resolution (e.g., value back to baseline value) or stabilization or until final database lock. If necessary, in order to obtain additional information to ensure safety to the subject, additional blood and urine samples may be taken at the discretion of the Investigator. Certain long-term AEs related to therapy cannot be followed until resolution within the setting of this study. In these cases follow-up will be the responsibility of the treating physician.

8.21. Reporting Requirements

Each AE is to be assessed to determine if it meets the criteria for SAEs. If an SAE occurs, expedited reporting will follow local and international regulations, as appropriate.

8.21.1. Serious Adverse Event Reporting Requirements

All SAEs independent of the circumstances or suspected cause must be reported on a SAE Form by the Investigator to:

The CRO within 24h of their knowledge of the event, preferably by fax or by e-mail. Contact information will be provided in a separate document.

The SAE form should include a clearly written narrative describing signs, symptoms, and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.

Follow-up and outcomes should be reported for all subjects who experience an SAE.

It is critical that the information provided on the SAE Form matches the information recorded in the source documents and in the eSource system for the same event.

Copies of additional laboratory tests, consultation reports, postmortem reports, hospital case reports, autopsy reports, and other documents should be sent when requested and applicable. Follow-up reports relative to the subject's subsequent course must be submitted to the CRO until the event has subsided or, in case of permanent impairment, until the condition stabilizes.

If an SAE occurs, Eloxx Pharmaceuticals is to be notified within 24 hours of Investigator awareness of the event by the CRO.

As noted in the Protocol-Specified SAE section, should an Investigator judge one of the identified protocol-specified SAEs to have a causal relationship with the investigational product, the event must be reported to the sponsor within 24 hours of Investigator awareness, even if that event is a component of the endpoint.

In particular, if the SAE is fatal or life-threatening, notification to Eloxx Pharmaceuticals must be made immediately, irrespective of the extent of available AE information. This time frame also applies to additional new information (follow-up) on previously forwarded SAE reports as well as to the initial and follow-up reporting of EDP, exposure via breastfeeding, and occupational exposure cases.

In the rare event that the Investigator does not become aware of the occurrence of an SAE immediately (eg, if an outpatient study subject initially seeks treatment elsewhere), the Investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the AE.

8.21.2. Nonserious Adverse Event Reporting Requirements

All AEs will be reported on the AE page(s) of the CRF. It should be noted that the form for collection of SAE information is not the same as the AE CRF. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same AE term should be used on both forms. AEs should be reported using concise medical terminology on the CRFs as well as on the form for collection of SAE information.

8.21.3. Sponsor's Reporting Requirements to Regulatory Authorities

Adverse events reporting, including suspected unexpected serious adverse reactions, will be carried out in accordance with applicable local regulations.

The CRO assumes responsibility for appropriate reporting of AEs to the regulatory authorities. the CRO will also report to the Investigator all SAEs that are unlisted (unexpected) and associated with the use of the drug. The Investigator (or the CRO where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol, unless otherwise required and documented by the IEC/IRB.

Adverse events reporting, including SUSARs will be carried out in accordance with applicable local regulations.

After termination of the clinical study (determined as LPLV), any unexpected safety issue that changes the risks benefit analysis and is likely to have an impact on the subjects who have participated in the study, together with proposed actions, will be reported by the Sponsor to the competent authority(ies) concerned as soon as possible.

9. STATISTICAL METHODS

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a statistical analysis plan (SAP).

9.1. Sample Size Determination

No formal sample size was estimated. The number of 9 subjects included in each dose group is deemed sufficient to respond to exploratory safety/tolerability and PK purposes.

Statistical analyses will be performed using SAS® v9.2 or higher (SAS Institute, Cary NC, USA), PK parameters calculation will be performed using Phoenix 6.2 (Pharsite Corp., USA) or later.

Descriptive statistics will be used to summarize demographics, baseline characteristics, and safety data.

9.2. Pharmacokinetic Analysis

Pharmacokinetic parameters of ELX-02 (C_{max}, t_{max}, AUC_t, AUC_{24h}, AUC_{48h}, AUC_{72h}, AUC_{inf}, $t_{1/2}$, Vd/F, and CL/F,) will be summarized by descriptive statistics by dose group and day of administration (sample size, mean, median, standard deviation, CV%, minimum and maximum, geometric mean and geometric CV%, when applicable).

Steady-state achievement will be evaluated by visual inspection of predose (and 1h postdose) concentrations figures.

For each of the PK parameters C_{max} and AUCs, the dose linearity will be assessed using a power model, including the log-transformed PK parameters as dependent variables and the log-transformed dose, day and dose*day as fixed effects. The slope for log-transformed dose (ß) will be estimated with its 90% confidence interval to examine dose proportionality. The t_{max} will be compared between dose groups and days using the appropriate non-parametric tests.

Plots of individual subject time-course profiles of ELX-02 after the administration will be presented by subject and day of administration in linear and semi-logarithmic scales. Figures enclosing all subjects for each dose and day of administration will be provided. Mean (SD) plasma concentrations obtained for each administration mode and drug dose will be plotted on

linear and semi-logarithmic scales. Through (predose and 1h postdose) figures will also be presented individually and as average.

The following parameters will be calculated based on ELX-02 urine concentrations: urinary excretion (Ae) of ELX-02 (in mass and %dose - fe) for each collection interval, cumulated urinary excretion (in %dose) for each collection interval, total excretion (in %dose) and renal clearance (using the AUC and Ae for the same time interval).

Actual sampling times will be used for PK analysis (when actual sampling times are not available, planned times will be used instead).

PK analysis will be performed on the PK population (i.e. enrolled subjects, with analyzable PK data and without relevant deviation interfering with the PK evaluations). The PK parameters will be calculated with WinNonlin Phoenix 6.2 (or later) software using non-compartmental analysis method.

Pharmacokinetic calculations will be based on individual subject plasma concentrations over time graphs. Samples with plasma concentrations below the LLOQ at early time-points will be set to as zero. Plasma concentrations below the LLOQ during the terminal phase will be omitted from the analysis.

9.3. Safety Analysis

Adverse events will be classified by system-organ classes and preferred terms and then summarized by number and percentage of volunteers experiencing AEs.

Summary tables will be generated for Auditory and Vestibular assessment data. Degree of hearing loss, and any abnormalities detected in vestibular assessments will be summarized by treatment and time point.

SRT, pure tone audiometry and tympanometry as measurements of auditory function and the ENT physical exam and vestibular assessments, in terms of absolute values and the changes from baseline will be summarized by treatment and time point with descriptive statistics.

Questionnaire data will be summarized, but will not be used to determine study outcomes.

9.4. Interim Analysis

No interim analysis is planned for this study but interim blinded PK analyses are planned after each cohort.

10. ETHICS

10.1. Institutional Review Board/Ethics Committee

It is the responsibility of the Investigator to have prospective approval of the study protocol, protocol amendments, informed consent documents, and other relevant documents, e.g., recruitment advertisements, if applicable, from the IRB/EC. All correspondence with the IRB/EC should be retained in the Investigator file. Copies of IRB/EC approvals should be forwarded to Eloxx Pharmaceuticals.

The only circumstance in which an amendment may be initiated prior to IRB/EC approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the Investigator must notify the IRB/EC and Eloxx Pharmaceuticals in writing immediately after the implementation.

An IRB/IEC should safeguard the rights, safety, and well-being of all study subjects. Special attention should be paid to studies that may include vulnerable subjects.

Before the start of the study, the Investigator (or Sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents:

- final protocol and, if applicable, amendments;
- Sponsor-approved ICF (and any updates or any other written materials to be provided to the subjects);
- Sponsor-approved subject recruiting materials;
- Investigator Brochure (or equivalent information) and addenda;
- available safety information;
- information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable;
- Investigator's current curriculum vitae or other documentation evidencing qualifications (unless not required, as documented by the IEC/IRB);
- information regarding funding, name of the Sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects;
- any other documents that the IEC/IRB may require to fulfill its obligation.

This study will be undertaken only after the IEC/IRB has given full written approval of the final protocol and amendments (if any), the ICF(s) and updates (if any), applicable recruiting materials, and any other written information to be provided to the subjects, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study, the Investigator (or Sponsor where required) will send the following documents and updates to the IEC/IRB for its review and approval, where appropriate:

- protocol amendments;
- revision(s) to the ICF and any other written materials to be provided to the subjects;
- new or revised subject recruiting materials approved by the Sponsor;

- revisions to compensation for study-related injuries or payment to subjects for participation in the study;
- Investigator's Brochure addenda or new edition(s);
- summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually);
- reports of AEs that are serious, unlisted, and associated with the IMP;
- new information that may adversely affect the safety of the subjects or the conduct of the study;
- deviations from or changes to the protocol to eliminate immediate hazards to the subjects;
- report of death of any subjects under the Investigator's care;
- notification if a new Investigator is responsible for the study at the CPU;
- Development Safety Update Report, Short-Term Study Specific Safety Summary and Line Listings, where applicable;
- any other requirements of the IEC/IRB.

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to the study subjects. If a deviation from or a change to the protocol was implemented to eliminate an immediate hazard to study subjects, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IEC/IRB as soon as possible.

The Investigator (or Sponsor where required) will notify the IEC/IRB about the study completion within 90 days after the end of the study (LPLV).

10.2. Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), Guidelines for GCP (ICH 1996 and E6 R2 revisions), and the Declaration of Helsinki (World Medical Association).

In addition, the study will be conducted in accordance with the protocol, the ICH guideline on GCP, and applicable local regulatory requirements and laws.

The Investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the study, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae or other relevant documentation requested by the Sponsor, the IRB/IEC, or the regulatory authority(ies).

10.3. Subject Information and Consent

All parties will ensure protection of subject personal data and will not include subject names or other identifiable data in any reports, publications, or other disclosures, except where required by law. The use of Subject initials should be avoided.

When study data are compiled for transfer to Eloxx Pharmaceuticals and other authorized parties, subject names, addresses, and other identifiable data will be replaced by a numerical code based on a numbering system provided by Eloxx Pharmaceuticals in order to de-identify study subjects. The study site will maintain a confidential list of subjects who participated in the study, linking each subject's numerical code to his or her actual identity. In case of data transfer, Eloxx Pharmaceuticals will maintain high standards of confidentiality and protection of subjects' personal data consistent with applicable privacy laws.

The informed consent documents must be in compliance with ICH GCP E6 R2, local regulatory requirements, and legal requirements, including applicable privacy laws.

The informed consent documents used during the informed consent process must be reviewed and approved by the sponsor, approved by the IRB/EC before use, and available for inspection.

Potential subjects will be fully informed of the nature of the study and of the risks and requirements of the study before any study-related assessment will be carried out. During the study, subjects will be given any new information that may affect their decision to continue participation. They will be informed that their participation in the study is voluntary and that they may withdraw from the study at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential AEs of the study, and who provide their consent voluntarily will be enrolled in the study.

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the Sponsor and the reviewing IEC/IRB.

Finally, they will be told that the Investigator will maintain a subject identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access, and agrees to allow his or her study physician to recontact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The language about the study used in the oral and written information, including the ICF, should be non-technical and practical and should be understandable to the subject (or the subject's legally acceptable representative). The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained consent, a copy of the ICF must be given to the subject.

The collection and processing of personal data from subjects enrolled in the study will be limited to those data that are necessary to investigate the safety, quality, and utility of the study drug used in the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data need to agree to keep the identity of the study subjects confidential.

The informed consent obtained from the subjects includes explicit consent for the processing of personal data and for the Investigator to allow direct access to subjects' original medical records for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

10.4. Subject Recruitment

Advertisements approved by IRBs/ECs and Investigator databases may be used as recruitment procedures.

Eloxx Pharmaceuticals will have an opportunity to review and approve the content of any study recruitment materials directed to potential study subjects before such materials are used.

10.5. Reporting of Safety Issues and Serious Breaches of the Protocol or ICH GCP

In the event of any prohibition or restriction imposed (ie, clinical hold) by an applicable competent authority in any area of the world, or if the Investigator is aware of any new information that might influence the evaluation of the benefits and risks of the investigational product, Eloxx Pharmaceuticals should be informed immediately.

In addition, the Investigator will inform Eloxx Pharmaceuticals immediately of any urgent safety measures taken by the Investigator to protect the study subjects against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the Investigator becomes aware of.

11. ADMINISTRATIVE REQUIREMENTS

11.1. Protocol Amendments

Neither the Investigator nor the Sponsor will modify this protocol without a formal amendment. All protocol amendments must be issued by the Sponsor and signed and dated by the Investigator. Protocol amendments must not be implemented without prior IEC/IRB approval nor when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazard to the subjects, in which case an amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the Investigator and IEC/IRB must be provided to the Sponsor or his designee. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

11.2. Subject Identification, Enrollment, and Screening Logs

The Investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the Sponsor site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the Investigator in the study file. To ensure subject confidentiality, no copies will be made. All reports and communications related to the study will identify subjects by initials and/or assigned number only.

The Investigator must also complete a subject screening log which reports on all subjects who were seen to determine eligibility for inclusion in the study.

11.3. Source Documentation

This study will utilize an electronic data capturing and information management system that will also serve as an eSource system for this study. The system combines all aspects of source data capturing with process control and clinical study management. All clinical and laboratory data, except those that are paper-based, will be collected directly in the system. The Source Document Identification Overview will specify which information will be eSource and which will be paper-based. The monitor will check data at the monitoring visits to the CPU. The Investigator will ensure that the data collected are accurate, complete, and legible. Data will be monitored within LabPas by the study monitor who has only reading rights. Any changes required following monitoring will be made by site personnel or the Investigator and will be documented with a full audit trail within the system.

At a minimum, source documentation must be available for the following: subject identification, eligibility, and study identification; date of informed consent, dates of visits, results of safety and efficacy parameters as required by the protocol, record of all AEs, follow-up of AEs, concomitant medication, drug receipt/dispensing/return records, study drug administration information, laboratory and ECG printouts (if not available digitally), date of study completion, and reason for early discontinuation of study drugs or withdrawal from the study, if applicable.

It is recommended that the author of an entry in the (e)Source documents be identifiable.

Source data may be directly captured from devices, transferred from third parties (e.g., laboratory data), or entered manually into the eSource system in use at the CPU. In such case, the majority of the source data will only be available electronically. The remainder of the data, captured initially on paper, may be entered retrospectively into the eSource system.

Following the ICH-GCP guidelines, direct access to (e)Source documentation (medical records) must be allowed.

11.4. Case Report Form Completion

All source data, except those that are paper-based, will be collected directly into the eSource system. Paper-based source data will be manually transcribed to the eSource system. Only the data required for the clinical database will be transferred electronically from the eSource system to the clinical database.

11.5. Monitoring

The monitoring of the study will be done under the responsibility of the Sponsor by the CRO.

The monitor will perform on-site monitoring visits as frequently as necessary. The monitor will record the dates of the visits in a study site visit log that will be kept at the CPU. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data captured in the eSource system for completeness and accuracy and perform data source verification to any data that has been captured as paper source or entered in the system later on. The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eSource system are known to the Sponsor and clinical staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the clinical staff.

Direct access to (e)Source documentation (medical records) must be allowed at all times for the purpose of verifying that the data recorded in the eSource system are consistent with the original (e)Source data. Findings from this review of the captured data will be discussed with the clinical staff. During on-site monitoring visits (notified and agreed upfront with the clinical staff), the relevant clinical staff will be available, the (e)Source documentation will be accessible, and a suitable environment for review of study-related documents will be provided. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

11.6. Data Management

Data management of the study will be performed under the responsibility of the Sponsor by the CRO.

After the data entered in the eSource system are released by the Investigator, the data will be uploaded into the clinical database to perform cleaning activities. Computerized data cleaning checks will be used in addition to manual review, including listings review, to check for discrepancies and to ensure consistency and completeness of the data. Queries emerging during data cleaning will be generated by the clinical data manager in the eSource system. The Investigator or his designee will answer the queries and update the source data, if needed.
The clinical database will be locked as soon as it is considered clean. Before the clinical database will be locked, the study eSource system will be locked by the clinical staff. Only

authorized and well-documented updates to the study data are possible after database lock. The locked database is used in the final statistical analysis for study reporting. Measures will be undertaken to protect subject data handed over by the Investigator to the data management department and during inspections against disclosure to unauthorized third parties. Subject confidentiality will be maintained at all times.

11.7. Data Quality Assurance

The accuracy and reliability of the study data will be assured by the selection of qualified Investigators and appropriate study sites, review of protocol procedures with the Investigator and associated personnel prior to the study, and by periodic monitoring visits by the Sponsor or designate.

Written instructions will be provided for the collection, preparation, and shipment of plasma samples. The Sponsor or his designee will review the eSource system for accuracy and completeness during (on-site) monitoring visits and after transmission to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. After upload of the data into the clinical study database, their accuracy verified using appropriate validation programs.

In accordance with Good Clinical Research Practice Guidelines and Recommendations, the Sponsor will be entitled to audit the facilities used in the clinical and laboratory parts of the study, as well as to access all the data files pertaining to the study. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

11.8. On-Site Audits

Representatives of the Sponsor's clinical quality assurance department may visit the CPU at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the eSource system. Subject privacy must, however, be respected. The Investigator and clinical staff are to be present and available for consultation during routinely scheduled site audit visits conducted by the Sponsor or his designee.

Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

11.9. Study Termination

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the IRB/EC, or investigational product safety problems, or at the discretion of Eloxx Pharmaceuticals. In addition, Eloxx Pharmaceuticals retains the right to discontinue development of the study drug at any time.

If a study is prematurely terminated or discontinued, Eloxx Pharmaceuticals will promptly notify the Investigator. After notification, the Investigator must contact all participating

subjects and the site pharmacy within 15 days. As directed by Eloxx Pharmaceuticals, all study materials must be collected and the database must be completed to the greatest extent possible.

In case of an early termination of the study for safety reasons, or temporary halt by the Sponsor, the IEC/IRB should be notified within 15 calendar days and should be provided with a detailed written explanation for the termination/halt.

An end-of-study declaration will be submitted to the regulatory authorities and IEC/IRB after the complete study has ended. This notification will be submitted within 90 days after the end of the study.

End of trial in a Member State of the European Union is defined as the time at which it is deemed that a sufficient number of subjects have been recruited and completed the study as stated in the regulatory application (ie, clinical trial application [CTA]) and ethics application in the Member State. Poor recruitment (recruiting less than the anticipated number in the CTA) by a Member State is not a reason for premature termination but is considered a normal conclusion to the study in that Member State.

11.10. Record Retention

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all eSource and all paper source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an 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 drug. These documents will be retained for a longer period if required according to the applicable regulatory requirements or per agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

If the responsible Investigator retires, relocates, or for any other reasons withdraws from his responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents without having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation related to the study, the Investigator must permit access to such reports.

11.11. Use of Information and Publication

11.11.1. Communication of Results by Eloxx Pharmaceuticals

Eloxx Pharmaceuticals fulfills its commitment to publicly disclose clinical trial results through posting the results of studies on www.clinicaltrials.gov (ClinicalTrials.gov), the European Clinical Trials Database (EudraCT), and other public registries in accordance with applicable local laws/regulations.

In all cases, study results are reported by Eloxx Pharmaceuticals in an objective, accurate, balanced, and complete manner and are reported regardless of the outcome of the study or the country in which the study was conducted.

Primary completion date is defined as the date that the final subject was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical study concluded according to the prespecified protocol or was terminated.

The results of the study will be reported in a Clinical Study Report generated under the responsibility of the Sponsor. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating Investigator.

Clinical narratives may be written for the following events (for example):

- All deaths (irrespective of drug relationship)
- All other SAEs during treatment with the study drugs
- All discontinuations of the study drugs due to AEs (irrespective of drug relationship)
- At the discretion of the team and after statistical analysis of the data, certain discontinuations not related to AEs or treatment failure, i.e., related to lost to follow-up or withdrawal of consent (irrespective of treatment group).
- Any events of special interest explicitly requested by the regulatory agencies

The coordinating/principal Investigator will sign off the final version of the Clinical Study Report. A summary of this final version will be provided to the Investigators, the applicable regulatory authorities, and the IECs/IRBs, if required by the applicable regulatory requirements, within 1 year after the end of the study (LPLV).

EudraCT

Eloxx Pharmaceuticals posts European (EU) Basic Results on EudraCT for all Eloxx Pharmaceuticals-sponsored interventional studies that are in scope of EU requirements. EU Basic Results are submitted for posting within 1 year of the primary completion date for studies in adult populations or within 6 months of the primary completion date for studies in pediatric populations.

11.11.2. Publications by Investigators

Eloxx Pharmaceuticals supports the exercise of academic freedom and has no objection to publication by the PI of the results of the study based on information collected or generated by the PI, whether or not the results are favorable to the Eloxx Pharmaceuticals product. However, to ensure against inadvertent disclosure of confidential information or unprotected inventions, the Investigator will provide Eloxx Pharmaceuticals an opportunity to review any proposed publication or other type of disclosure of the results of the study (collectively, “Publication”) before it is submitted or otherwise disclosed.

The Investigator will provide any publication to Eloxx Pharmaceuticals at least 30 days before they are submitted for publication or otherwise disclosed. If any patent action is required to protect intellectual property rights, the Investigator agrees to delay the disclosure for a period not to exceed an additional 60 days.

The Investigator will, on request, remove any previously undisclosed confidential information before disclosure, except for any study- or Eloxx Pharmaceuticals product-related information necessary to the appropriate scientific presentation or understanding of the study results.

If the study is part of a multicenter study, the Investigator agrees that the first publication is to be a joint publication covering all study sites, and that any subsequent publications by the PI will reference that primary publication. However, if a joint manuscript has not been submitted for publication within 12 months of completion or termination of the study at all participating sites, the Investigator is free to publish separately, subject to the other requirements of this section.

For all publications relating to the study, the institution will comply with recognized ethical standards concerning publications and authorship, including Section II - "Ethical Considerations in the Conduct and Reporting of Research" of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, http://www.icmje.org/index.html#authorship, established by the International Committee of Medical Journal Editors.

Publication of study results is also provided for in the clinical study agreement (CSA) between Eloxx Pharmaceuticals and the institution. In this section entitled Publications by Investigators, the defined terms shall have the meanings given to them in the CSA.

If there is any conflict between the CSA and any Attachments to it, the terms of the CSA control. If there is any conflict between this protocol and the CSA, this protocol will control as to any issue regarding treatment of study subjects, and the CSA will control as to all other issues.

11.12. Registration of Clinical Studies and Disclosure of Results

The Sponsor will register the existence of a clinical study and disclose its results as required by law.

11.13. Confidentiality

All study documents are provided by the Sponsor to the Investigator and appointed clinical staff in confidence. None of this material may be disclosed to any party not directly involved in the study without the Sponsor's written permission.

The Investigator must assure that subjects' anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the subjects' study numbers, names, addresses, and telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from the Sponsor.

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13. APPENDIX

13.1. Site Reactions to Injections and Infusions DAIDS Grading Table

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 one*	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 & functional activities	Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness *Report only one* *>15 years of age*	2.5 to < 5 cm in diameter OR 6.25 to < 25 cm2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities	≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities	≥ 10 cm in diameter OR ≥ 100 cm2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one* *>15 years of age*	Same as for **Injection Site Erythema or Redness**	Same as for **Injection Site Erythema or Redness**	Same as for **Injection Site Erythema or Redness**	Same as for **Injection Site Erythema or Redness**
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 & functional activities	NA

13.2. Dizziness Handicap Inventory

P1. Does looking up increase your problem?	o Yes o Sometimes o No
E2. Because of your problem, do you feel frustrated?	o Yes o Sometimes o No
F3. Because of your problem, do you restrict your travel for business or recreation?	o Yes o Sometimes o No
P4. Does walking down the aisle of a supermarket increase your problems?	o Yes o Sometimes o No
F5. Because of your problem, do you have difficulty getting into or out of bed?	o Yes o Sometimes o No
F6. Does your problem significantly restrict your participation in social activities, such as going out to dinner, going to the movies, dancing, or going to parties?	o Yes o Sometimes o No
F7. Because of your problem, do you have difficulty reading?	o Yes o Sometimes o No
P8. Does performing more ambitious activities such as sports, dancing, household chores (sweeping or putting dishes away) increase your problems?	o Yes o Sometimes o No
E9. Because of your problem, are you afraid to leave your home without having someone accompany you?	o Yes o Sometimes o No
E10. Because of your problem have you been embarrassed in front of others?	o Yes o Sometimes o No
P11. Do quick movements of your head increase your problem?	o Yes o Sometimes o No
F12. Because of your problem, do you avoid heights?	o Yes o Sometimes o No
P13. Does turning over in bed increase your problem?	o Yes o Sometimes o No
F14. Because of your problem, is it difficult for you to do strenuous homework or yard work?	o Yes o Sometimes o No
E15. Because of your problem, are you afraid people may think you are intoxicated?	o Yes o Sometimes o No
F16. Because of your problem, is it difficult for you to go for a walk by yourself?	o Yes o Sometimes o No
P17. Does walking down a sidewalk increase your problem?	o Yes o Sometimes o No
E18.Because of your problem, is it difficult for you to concentrate	o Yes o Sometimes o No

F19. Because of your problem, is it difficult for you to walk around your house in the dark?	○ Yes ○ Sometimes ○ No
E20. Because of your problem, are you afraid to stay home alone?	○ Yes ○ Sometimes ○ No
E21. Because of your problem, do you feel handicapped?	○ Yes ○ Sometimes ○ No
E22. Has the problem placed stress on your relationships with members of your family or friends?	○ Yes ○ Sometimes ○ No
E23. Because of your problem, are you depressed?	○ Yes ○ Sometimes ○ No
F24. Does your problem interfere with your job or household responsibilities?	○ Yes ○ Sometimes ○ No
P25. Does bending over increase your problem?	○ Yes ○ Sometimes ○ No

DHI Scoring Instructions

The Subjects will be asked to answer each questions as it pertains to dizziness or unsteadiness problems, specifically considering their condition during the last month. Questions are designed to incorporate functional (F), physical (P), and emotional (E) impacts on disability.

To each item, the following scores can be assigned:

- No=0
- Sometimes=2
- Yes=4

Scores :

- >10 points should be referred to balance specialists for further evaluation.
- 16-34 points (mild handicap)
- 36-52 points (moderate handicap)
- 54 + points (severe handicap)

13.3. Tinnitus Handicap Inventory

INSTRUCTIONS: The purpose of this questionnaire is to identify difficulties that you may be experiencing because of your tinnitus. Please answer every question. Please do not skip any questions.

1. Because of your tinnitus, is it difficult for you to concentrate?	Yes	Sometimes	No
2. Does the loudness of your tinnitus make it difficult for you to hear people?	Yes	Sometimes	No
3. Does your tinnitus make you angry?	Yes	Sometimes	No
4. Does your tinnitus make you feel confused?	Yes	Sometimes	No
5. Because of your tinnitus, do you feel desperate?	Yes	Sometimes	No
6. Do you complain a great deal about your tinnitus?	Yes	Sometimes	No
7. Because of your tinnitus, do you have trouble falling to sleep at night?	Yes	Sometimes	No
8. Do you feel as though you cannot escape your tinnitus?	Yes	Sometimes	No
9. Does your tinnitus interfere with your ability to enjoy your social activities (such as going out to dinner, to the movies)?	Yes	Sometimes	No
10. Because of your tinnitus, do you feel frustrated?	Yes	Sometimes	No
11. Because of your tinnitus, do you feel that you have a terrible disease?	Yes	Sometimes	No
12. Does your tinnitus make it difficult for you to enjoy life?	Yes	Sometimes	No
13. Does your tinnitus interfere with your job or household responsibilities?	Yes	Sometimes	No
14. Because of your tinnitus, do you find that you are often irritable?	Yes	Sometimes	No
15. Because of your tinnitus, is it difficult for you to read?	Yes	Sometimes	No
16. Does your tinnitus make you upset?	Yes	Sometimes	No

17. Do you feel that your tinnitus problem has placed stress on your relationships with members of your family and friends?	Yes	Sometimes	No
18. Do you find it difficult to focus your attention away from your tinnitus and on other things?	Yes	Sometimes	No
19. Do you feel that you have no control over your tinnitus?	Yes	Sometimes	No
20. Because of your tinnitus, do you often feel tired?	Yes	Sometimes	No
21. Because of your tinnitus, do you feel depressed?	Yes	Sometimes	No
22. Does your tinnitus make you feel anxious?	Yes	Sometimes	No
23. Do you feel that you cannot longer cope with your tinnitus?	Yes	Sometimes	No
24. Does your tinnitus get worse when you are under stress?	Yes	Sometimes	No
25. Does your tinnitus make you feel insecure?	Yes	Sometimes	No

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Total Per Column				
	x4	**x2**	**x0**	
Total score		+	+	=

GRADE	SCORE	DESCRIPTION
1	0-16	Slight: Only heard in quiet environment, very easily masked. No interference with sleep or daily activities.
2	18-36	Mild: Easily masked by environmental sounds and easily forgotten with activities. May occasionally interfere with sleep but not daily activities.
3	38-56	Moderate: May be noticed, even in the presence of background or environmental noise, although daily activities may still be performed.
4	58-76	Severe: Almost always heard, rarely, if ever, masked. Leads to disturbed sleep pattern and can interfere with ability to carry out normal daily activities. Quiet activities affected adversely.
5	78-100	Catastrophic: Always heard, disturbed sleep patterns, difficulty with any activity.

13.4. Document History

Summary of Changes: Protocol ELX-02 Dated 8 NOV 2018 to Protocol ELX-02 Dated 26 February 2019

Throughout, weight has been added to each time the subject checks into the clinic.

Check in time to CPU of 7:30 AM has been deleted to accommodate the new CROs standards.

Throughout, description of vital sign and ECG procedure has been clarified to indicate that the measurement should be taken after 5 minutes in the supine position.

Throughout, a(n) highly effective method of contraception has been replaced with 2 highly effective methods of contraception.

Throughout, gravity has been changed to specific gravity.

Throughout, urine pregnancy test will be performed on all women, regardless of childbearing potential.

Throughout, water consumption prior to dosing has been changed from 500 mL to at least 500 mL to allow for flexibility.

Throughout, one glass of water had been clarified to indicate that the volume will be approximately 250 mL.

Throughout, SGS Life Sciences (SGS) has been replaced with “the CRO”, to reflect the change in the CRO.

Phone and FAX numbers for the CRO have been replaced with “contact information will be provided in a separate document”, to reflect the change in the CRO.

Additional minor modifications grammatical, punctuation, and wording modifications have been made to provide clarification.

Section	**Used to Read**	**Now Reads**	**Rationale for Change**
Header Date changed	8 NOV 2018	26 FEB 2019	Changed to reflect new version
Title Page	Investigator: To be determined	Azra Hussaini, MD PAREXEL International Baltimore Early Phase Clinical Unit	New investigator added from the US

Section	Used to Read	Now Reads	Rationale for Change
		Medstar Harbor Hospital, 7th floor 3001 South Hanover Street Baltimore, MD, 21225 USA	
Signature of Sponsor Representative page	Ajay Aggarwal, MD	Greg Williams, PhD, Chief Operating Officer	Change in personnel at Eloxx
Signature of Investigator		Azra Hussaini, MD	New US investigator added
PROTOCOL SUMMARY Number of Subjects	Up to 63 healthy adult subjects will participate in the study: 9 in each cohort randomized 2:1 to ELX-02 and placebo. Both males and females need to be enrolled in each cohort, a significant number of female subjects need to be enrolled. Additional cohorts or subjects may be added to reaffirm safety of ELX-02.	Up to 63 healthy adult subjects will participate in the study: 9 in each cohort randomized 2:1 to ELX-02 and placebo. Both males and females need to be enrolled in each cohort~~, a significant number of female subjects need to be enrolled~~. Additional cohorts or subjects may be added to reaffirm safety of ELX-02.	Changed to correct an error
PROTOCOL SUMMARY and **4.1. Inclusion Criteria** Inclusion Criteria #8	Normal renal function (glomular filtration rate >60 mL/min) based on creatinine plasma concentration and the Modification of Diet in Renal Disease (MDRD) equation for estimated glomular filtration rate.	Normal renal function (***glomerular*** filtration rate >60 mL/min***/1.73m²***) based on creatinine plasma concentration and the Modification of Diet in Renal Disease (MDRD) equation for estimated ***glomerular*** filtration rate.	Correction of error, spelling of glomerular corrected, units added

Section	Used to Read	Now Reads	Rationale for Change
PROTOCOL SUMMARY and **4.1. Inclusion Criteria** Inclusion Criteria #12	Body Mass Index (BMI) of 19.0 to 30 kg/m2 (inclusive); and a toal body weight of >50.0 kg (110 lbs) and <100.0 kg	Body Mass Index (BMI) of 19.0 to ~~30~~ 32 kg/m^2 (inclusive); and a toal body weight of >50.0 kg (110 lbs) and <100.0 kg	Changed to facilitate enrollment
Table 1: Time and Events Schedule **7. STUDY PROCEDURES** Additional timepoints also added to this section	Urine drugs of abuse Screening Day -1 Day 28	Urine drug screen Day -1, Day 7, Day 14, Day 21, and Day 28. (UDS) Screening Day -1 ***Day 7*** ***Day 14*** ***Day 21*** Day 28	Change in nomenclature Additional timepoints for assessment added
Table 1: Time and Events Schedule **PROTOCOL SUMMARY** **7. STUDY PROCEDURES** Change in text also added to this section	Alcohol breath test	Serum alcohol test Day -1, Day 7, Day 14, Day 21, and Day 28.	Test changed at the request of the CRO Additional timepoints for assessment added
Table 1: Time and Events Schedule **Section 6.5.1. Female Subjects** **PROTOCOL SUMMARY** **7. STUDY PROCEDURES** Change in text also added to this section	Female subjects of non-child bearing potential must agree to undergo pregnancy test at screening.	All ~~F~~female subjects ~~of non-child bearing potential~~ regardless of childbearing potential, must agree to undergo pregnancy test at screening***, and on Day -1, Day 7, Day 14, Day 21, Day 28 and Day 36***.	Additional timepoints for assessment added
Table 1: Time and Events Schedule Footnote i.	Follow up testing will be performed at Day 3 (-1 day), Day 10 (-1 day), Day 17 (-1 day), Day 24 (-1 day), and EOS (7-11 days after last dosing).	Follow up testing will be performed at Day 3 (-1 day), Day 10 (-***2*** day***s***), Day 17 (-***2*** day***s***), Day 24 (-***2*** day***s***),	Windows changed to allow more flexibility for CRO

Section	Used to Read	Now Reads	Rationale for Change
		and EOS (7-11 days after last dosing).	
STUDY ADMINISTRATIVE STRUCTURE	**CLINICAL RESEARCH ORGANIZATION** Information will be provided in a separate document **CLINICAL LABORATORY** ZNA Klinisch Laboratorium Campus Middelheim Lindendreef 1 2020 Antwerpen Belgium **EAR, NOSE, AND THROAT SPECIALIST** Guy Cools, MD ZNA Department Ear, Nose, and Throat (ENT) Campus Middelheim Lindendreef 1 2020 Antwerpen Belgium **CLINICAL MONITORING** SGS Life Sciences Project Manager: Marthe Heylen **PHARMACOVIGILANCE** SGS Life Sciences, Medical Affairs Department **BIOMETRICS** SGS Life Sciences Project Manager: Lisbet Reynders Biostatistician: Bastiaan Janssen Case Report Form (CRF) design, dta management, statistics and medical writing	Section deleted	Section deleted to reflect change in CRO, the information will be provided in a separate document
4. SUBJECT SELECTION 2nd sentence	Both males and females need to be enrolled in each cohort, a significant number of female subjects need to be enrolled.	Both males and females need to be enrolled in each cohort~~, a significant number of female subjects need to be enrolled~~.	Changed to correct an error

Section	Used to Read	Now Reads	Rationale for Change
4. SUBJECT SELECTION **Section 4.11. Removal, Replacement or Early Withdrawal** **Item #4**	Positive urine drugs of abuse and/or alcohol breath test on admission to the CPU on Day -1.	Positive ~~urine drugs of abuse~~ UDS and/or ***serum*** alcohol ~~breath~~ test on admission to the CPU on Day -1. ***If subjects have a positive UDS and/or serum alcohol test after Day -1, their continued participation will be re-evaluated on a case by case basis.***	Added to provide guidance due to the addition of drug/alcohol sceening tests.
4. SUBJECT SELECTION **Section 4.11. Removal, Replacement or Early Withdrawal** Additional stipulation added		***11. Subjects may be replaced at the discretion of the Sponsor.***	Clarification added
5. STUDY TREATMENTS 5.4 Administration 3rd paragraph, 1st sentence	The drug will be administered as SC injection(s) to the abdominal region around the umbilicus or to any area with a significant subcutaneous adipose tissue (e.g. thigh), with a maximum of 4 injections per dosing.	The drug will be administered as SC injection(s) to the abdominal region around the umbilicus or to any area with a significant subcutaneous adipose tissue (e.g. thigh)~~, with a maximum of 4 injections per dosing.~~	Clarified to correctly describe dosing options
6. STUDY RESTRICTIONS **6.3 Meals and Dietary Restrictions**		***- Subjects will abstain from smoking for 6 months prior to the first screening visit until 48h after the***	

Section	Used to Read	Now Reads	Rationale for Change
Two bullet points have been added		***last study drug administration.*** ***- Subjects will abstain from illicit drug use from the first screening visit until 48h after the last study drug administration.***	
6. STUDY RESTRICTIONS **6.5.1. Female Subjects**	All other female subjects (including females with tubal ligations) will be considered to be of childbearing potential and must use 1 highly effective….	***Unless willing to abstain from sexual intercourse for 14 days prior to the first study drug administration and for 28 days after the last dose, 2*** ~~1~~ highly effective….	Modified to correct inconsistancies between sections of the protocol Abstention has been deleted as a method of highly effective birth control due to rewording
6. STUDY RESTRICTIONS **6.5.2. Male Subjects** New sentence added		***Unless they are abstaining from sexual intercourse for 14 days days prior to the first study drug administration and for 28 days after the last dose…***	Modified to correct inconsistancies between sections of the protocol Abstention has been deleted as a method of highly effective birth control due to rewording
7. STUDY PROCEDURES 7.4.2. Urine Collection 2nd bullet, 2nd sentence AND 7th bullet, 2nd sentence	Each sample's weight will be recorded individually prior to removing urine for analyses cfr. the Time and Event Schedule (i.e., urinalysis, PK, biomarker, and creatinine)	Each sample's weight will be recorded individually prior to removing urine for ~~analyses cfr. the Time and Event Schedule (i.e., urinalysis, PK, biomarker, and creatinine)~~	Deleted to correct typographical error

Document	**Version Date**	**Summary of Changes *and Rationale***
Original protocol	Final dated 26 June 2017	Not applicable (N/A)
Revised protocol	Revised final, dated 18 July 2017	Following recommendations of the local (Belgian) health authorities the following tests/actions were added to the previous version of the protocol: a. Additional audiometric tests will be performed on days before dosing i.e., Day 3 (-1 day), Day 7 (-1 day), Day 10 (-1 day), Day 14 (-1 day), Day 17 (-1 day), Day 21 (-day), Day 24 (-1 day), and Day 28 (-1 day). b. The audiologist who will perform the audiometric tests will write his/her findings on an auditory entry form and send abnormal results to the adjudication committee (consisting of a physician and audiologist). The adjudication committee will decide if further dosing will occur, taken into account the stopping criteria. c. The data safety meeting board (DSMB) will meet after each cohort and will follow the recommendations of the adjudication committee. Vestibular questionnaires will not be performed on Day 15 due to the additional audiometric tests on Day 14.
Revised protocol, 25 Aug 2017	Revised final, dated 25 Aug 2017	Additional urine samples for creatinine safety evaluation will be taken at the following timepoints: Screening, Day -1, Day 1; 12h postdose, Day 2; 24h and 36h postdose, Day 3, Day 4, Day 8, Day 11, Day 15, Day 18, Day 22, Day 25, Day 28, Day 29; 12h postdose, Day 30; 24h and 36h postdose, Day 31, and Day 36 (same urine sampling timepoints as for the renal injury biomarkers). The predose urine samples for creatinine safety at Day 1 and Day 29 will remain. The vital signs at screening will be assessed supine following at least 5 min of supine rest instead of after standing for 3 min. During the treatment period the VS will be assessed following at least 5 min of supine rest as well.
Revised protocol, 27 Sep 2017	Revised protocol, 27 Sep 2017	Changes to the previous version:

Document	Version Date	Summary of Changes *and Rationale*
		• Respiratory rate was added as a vital sign that needs to be measured. • The amount of blood taken for the analysis of the housekeeping proteins per timepoint has been changed from '2 mL' to '2 x 8mL'. • Blood samples for determination of mitochondrial mutations testing will be taken using 2.7 mL tubes (instead of 2.5 mL tubes) containing K3EDTA. • The total volume of blood that will be drawn from each subject has been changed from approximately 160 mL for female and 155 mL for male subjects into 547 mL for female and 542 mL for male subjects. • Section 7.3.9: the following information was added: All blood samples for retention need to be taken after an overnight fast of at least 8h, except for the samples taken at 3h postdose on Day 1 and Day 29. Processing and storage of the samples are detailed in the laboratory manual provided by the Sponsor. All blood samples for safety assessments should be taken after an overnight fast of at least 8h, except for the samples taken at 6h postdose on Day 1 and Day 29. • Section 7.4.2 has been adapted (bold text has been added, strike through has been deleted): - Day 1: Predose **collection** (first void in the morning). **Urine samples for PK, biomarker, and creatinine analysis will be collected. Gravity will be calculated for the collection.** - Day 1 (0-12 hours postdose collection period): Subjects will be encouraged to void as frequently as possible (i.e. asked every hour if they can collect urine), and each individual sample will be collected separately. Each sample's weight will be recorded individually

Document	Version Date	Summary of Changes *and Rationale*
		prior to removing urine for analyses **cfr. the Time and Event Schedule (i.e., urinalysis, PK, biomarker, and creatinine)**. ~~After weighing and removing urine for analyses, the collection container with remaining urine may be discarded.~~ Gravity will be calculated for each ~~sample~~ **collection**. - Day 1: 12-24 hours postdose **collection** period. This urine collection period includes all voids starting after 12h and includes the first void on the next day (i.e., 24h postdose **void**). **The urine will be collected every 6h (i.e., 12-18h collection and 18-24h collection). The 12-18h voids and the 18-24h voids** ~~All voids during this period~~ will be collected into **separate** urine collection containers. **In addition, a single urine collection will be taken 24h after dosing using a third container (i.e., first morning void of Day 2).**~~However, the single 24h void (i.e. first morning void of Day 2) will be collected in a separate container.~~ **All** ~~Both~~ urine containers will be weighed individually and the ~~sum of both~~ weights will be recorded. Urine samples for urinalysis and biomarker analysis will be withdrawn from the single 24h urine collection. Urine samples for PK **and creatinine** analysis will be withdrawn from the **pooled 12-18h and pooled 18-24h** ~~combined, mixed 12-24h~~ **urine collection** containers. Gravity will be calculated for each ~~sample~~ **collection**. - Day 2 – Day 3: 24-48 hours postdose **collection** period. This urine collection period includes all voids subsequent to the **24h postdose void** ~~first morning void of Day 2~~ and includes the first void on the next day (i.e., 48 hours postdose **void**). **The urine will be collected every 12h (i.e. 24-36h collection and 36-48h collection).** ~~All voids during this period~~ **The 24-36h voids and the 36-48h voids** will be collected into **separate** ~~one~~ urine collection containers. **In addition, a single urine collection will be**

Document	Version Date	Summary of Changes *and Rationale*
		taken 36 hours after dosing using a third container and a single urine collection will be taken 48 hours after dosing using a fourth container (i.e., the first morning void of Day 3). ~~However, the single 48h void (i.e., first morning void of Day 3) will be collected in a separate container.~~ ~~Both~~ **All** urine containers will be weighed individually and ~~the sum of both~~ **the** weights will be recorded. Urine samples for urinalysis ~~and biomarker analysis~~ will be withdrawn from the single 48h urine void. **Urine samples for biomarker analysis will be withdrawn from the single 36h and 48h urine void.** Urine samples for PK **and creatinine** analysis will be withdrawn from the ~~combined, mixed~~ pooled 24-36h and pooled 36-48h urine collection containers. Gravity will be calculated for each **collection** ~~sample~~. - Day 3 – Day 4: 48-72 hours postdose **collection** period. This urine collection period includes all voids subsequent to the **48h postdose void** ~~first morning void of Day 3~~ and includes the first void on the next day (i.e., **72** ~~48~~ hours postdose **void**). ~~All voids during this period will be collected into one urine collection container.~~ During this collection period**,** subjects will be at home and collect the urine in urine jars (provided by the study site personnel). **During this period, voids will be collected into a urine collection container. In addition, a single urine collection will be taken 72 hours after dosing using a second container (i.e., the first morning void of Day 4). Urine samples for biomarker analysis will be withdrawn from the single 72h urine void. Urine samples for PK and creatinine analysis will be withdrawn from the pooled 48-72h urine collection container. Gravity will be calculated for each collection** ~~sample~~**.** - Day 29: Predose **collection** (first void in the morning). **Urine samples for PK, biomarker, and creatinine analysis will be collected. Gravity will be calculated for the collection.**

Document	Version Date	Summary of Changes *and Rationale*
		- Day 29 (0-12 hours postdose collection period): Subjects will be encouraged to void as frequently as possible (i.e. asked every hour if they can collect urine), and each individual sample will be collected separately. Each sample's weight will be recorded individually prior to removing urine for analyses **cfr. the Time and Event Schedule (i.e., urinalysis, PK, biomarker, and creatinine)**. ~~After weighing and removing urine for analyses, the collection container with remaining urine may be discarded.~~ Gravity will be calculated for each ~~sample~~ **collection**. - Day 29: 12-24 hours postdose **collection** period. This urine collection period includes all voids starting after 12h and includes the first void on the next day (i.e., 24h postdose **void**). **The urine will be collected every 6h (i.e., 12-18h collection and 18-24h collection). The 12-18h voids and the 18-24h voids** ~~All voids during this period~~ will be collected into **separate** urine collection containers. **In addition, a single urine collection will be taken 24h after dosing using a third container (i.e., first morning void of Day 30).**~~However, the single 24h void (i.e. first morning void of Day 2) will be collected in a separate container.~~ **All** ~~Both~~ urine containers will be weighed individually and the ~~sum of both~~ weights will be recorded. Urine samples for urinalysis and biomarker analysis will be withdrawn from the single 24h urine collection. Urine samples for PK **and creatinine** analysis will be withdrawn from the **pooled 12-18h and pooled 18-24h** ~~combined, mixed 12-24h~~ **urine collection** containers. Gravity will be calculated for each ~~sample~~ **collection**. - Day 30 – Day 31: 24-48 hours postdose **collection** period. This urine collection period includes all voids subsequent to the **24h postdose void** ~~first morning void of Day 2~~ and includes the first void on the next day (i.e., 48 hours postdose **void**).

Document	Version Date	Summary of Changes *and Rationale*
		The urine will be collected every 12h (i.e. 24-36h collection and 36-48h collection). ~~All voids during this period~~ **The 24-36h voids and the 36-48h voids** will be collected into **separate** ~~one~~ urine collection containers. **In addition, a single urine collection will be taken 36 hours after dosing using a third container and a single urine collection will be taken 48 hours after dosing using a fourth container (i.e., the first morning void of Day 31).** ~~However, the single 48h void (i.e., first morning void of Day 3) will be collected in a separate container.~~ ~~Both~~ **All** urine containers will be weighed individually and ~~the sum of both~~ **the** weights will be recorded. Urine samples for urinalysis ~~and biomarker analysis~~ will be withdrawn from the single 48h urine void. **Urine samples for biomarker analysis will be withdrawn from the single 36h and 48h urine void.** Urine samples for PK **and creatinine** analysis will be withdrawn from the ~~combined, mixed~~ pooled 24-36h and pooled 36-48h urine collection containers. Gravity will be calculated for each **collection** ~~sample~~. - Day 31 – Day 32: 48-72 hours postdose **collection** period. This urine collection period includes all voids subsequent to the **48h postdose void** ~~first morning void of Day 3~~ and includes the first void on the next day (i.e., **72** ~~48~~ hours postdose **void**). ~~All voids during this period will be collected into one urine collection container.~~ During this collection period**,** subjects will be at home and collect the urine in urine jars (provided by the study site personnel). **During this period, voids will be collected into a urine collection container. In addition, a single urine collection will be taken 72 hours after dosing using a second container (i.e., the first morning void of Day 32). Urine samples for biomarker analysis will be withdrawn from the single 72h urine void. Urine samples for PK and creatinine analysis will be withdrawn from the pooled**

Document	Version Date	Summary of Changes *and Rationale*
		48-72h urine collection container. Gravity will be calculated for each collection ~~sample~~**.**
Revised protocol	23 Jan 2018	Rationale for the amendment: During the conduct of the first cohort in this trial, audiological testing was performed at baseline, prior to each dose and at the end of the study. Bidirectional variability was observed inconsistent with ototoxicity, which can be attributed to the frequency of testing. After querying these results and reviewing them with the dedicated, independent, third-party Auditory and Vestibular Data Safety Monitoring Board (AVDSMB), Eloxx Pharmaceuticals believe that the frequency of testing subjects may be contributing to the variability by creating learning effects, burdening the site and subjects and reducing the careful attention needed for these listening tasks. The frequency of testing causes variability and does not safeguard the participating subjects. Therefore, upon the recommendation of the AVDSMB, Eloxx Pharmaceuticals has reduced the number of audiological tests during the study to once weekly. Changes to the previous version (additions are marked in bold, deletions are Strikethrough): Auditory and vestibular assesments: • Addition of baseline ENT exam for eligible subjects (in addition to the screening visit to confirm eligibility). During the screening period subjects will undergo auditory and vestibular assessments to confirm eligibility (Day -42 to -8). In addition, a baseline exam (PTA and HFA will be duplicated) will be performed prior to initial dosing (within 7 days prior to dose). There must be a minimum of 24 hours between the screening and baseline ENT exams • The SRT exam will be necessary at screening and bassline and for all other study SRT will be

Document	Version Date	Summary of Changes *and Rationale*
		performed only upon ASHA significant change criteria/ reverse ASHA significant change criteria observed in PTA/HFA. • Reducing the frequency of the follow up ENT at Day 3 (-1 day), Day 10 (-1 day), Day 17 (-1 day), Day 24 (-1 day) and EOS (7-11 days after last dosing). • Inclusion criteria 11 was changed as follows: "No personal history (or current) or hereditary hearing loss, persistent tinnitus, persistent vertigo, persistent imbalance, persistent unsteadiness **and no regular exposure to excessive noise (such as employment in a factory, music teacher, etc).** • Exclusion creteria 4 was modifed as follows: "Presence of mitochondrial mutations making subject susceptible to aminoglycoside toxicity ~~(A1555G, C1494T, T1095C, A827G, 1 BP DEL, 961T, C INS).~~ • Exclusion creteria 6 was modifed as follows: Subjects with any abnormality at screening, that indicates the presence of a vestibular pathology, conductive hearing loss or balance problem (by an ENT). Subjects with **audiometry** abnormalities in the **conventional frequencies (up to 8 kHz)** results at screening as follows: any pure-tone threshold >55 dB and/or inter-ear difference in any frequency of >20 dB. • Creatinine samples will be taken at all timepoints corresponding to renal injury biomarker collection. On days 1-4 and 29-32, samples for renal injury biomarkers and creatinine will be taken from urine spot samples at 24hr, 36hr, 48hr and 72hr post dose.

Document	Version Date	Summary of Changes *and Rationale*
		• Protocol Clarifications and Administrative changes were added to adjust study procedures and personnel changes • Urine protein and Albumin test were added to screening and EOS visits
Revised Protocol	15 May 2018	• Following recommendations of the local (Belgium) Health Authorities, a cohort for administration of a 5.0 mg/kg dose could be implemented in this MAD study after satisfactory safety results were obtained for this dose in the SAD study. Results from the SAD study evaluating the ELX-02 5.0 mg/kg and 7.5 mg/kg SC doses were added to Section 1.2.2. Given the favorable safety profile of the doses, a dose cohort of 5.0 mg/kg was added to this MAD study. • Glomerular filtration rate was only meant to be an inclusion criterion and was carried through the protocol inadvertently. Use of the eGFR estimated by the MDRD equation to monitor acute changes in renal function in a normal population is not appropriate and not indicated because the MDRD equation applies only to subjects with a GFR below 60 ml/min, can never be used to assess acute renal changes, and depends on an equation that smooths out many critical parameters. We are measuring serum creatinines and KIM-1 and clusterin as parameters of renal function. Therefore, assessment eGFR estimated by the MDRD equation was removed from visits after the screening visit. • Sponsor address was changed.
Revised Protocol	24 September 2018	• Injection site reactions (ISR) have been observed for all cohorts completed to date. The completed cohorts and number of ISR are as follows: 0.1 mg/kg (2 subjects with 2 ISR), 0.3 mg/kg (7 subjects with 10 ISR), 1.0 mg/kg (5 subjects with 41 ISR) and 2.5 mg/kg (7 subjects

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		with 72 ISR). The 0.1 and 0.3 mg/kg cohort ISR were of short duration (maximum 2 weeks) compared to the 1.0 and 2.5 mg/kg cohorts (currently available data indicate ISR lasting up to 63 days). The events were generally mild, but the cumulative number of events in the 2.5 mg/kg cohort limit tolerability. The 0.1 and 0.3 mg/kg cohorts (50 mg/mL) were tolerated better then the 1.0 and 2.5 mg/kg doses (100mg/mL). In the 2.5 mg/kg cohort, one subject had a high frequency hearing change that met ASHA criteria. Therefore, an additional cohort of 2.5 mg/kg or 1 mg/kg at lower concentration (50 mg/mL instead of 100 mg/mL) is proposed. Adjustment to further cohorts may also be proposed depending upon the outcome of the repeated cohort. • Additonal SC injection site locationa are being allowed (e.g. thigh) to spread out the injection sites. • Allowance for screening and baseline ENT exams to be performed on the same day to minimize subject burden

Section	Used to Read	Now Reads	Rationale for Change
Header Date changed	24 SEPT 2018	8 NOV 2018	Changed to reflect new version
Cover Page	A Phase 1, Randomized, Double-Blinded, Placebo-Controlled, Third Party Open, Multiple Dose Escalation Study to Evaluate the Safety, Tolerability and Pharmacokinetics of Subcutaneously Administered ELX-02 in Independent	A Phase 1, Randomized, Double-Blinded, Placebo-Controlled, Third Party Open, Multiple Dose Escalation ~~Single Center~~ Study to Evaluate the Safety, Tolerability and Pharmacokinetics of Subcutaneously Administered ELX-02 in Independent	Protocol title has changed to reflect addition of new investigator, title has been changed throughout as appropriate

Section	Used to Read	Now Reads	Rationale for Change
	Consecutive Cohorts of Healthy Subjects	Consecutive Cohorts of Healthy Subjects	
	N/A	*US IND Number: 137391*	US IND Number added to expand the study to the USA
	N/A	*To be determined, Investigator, USA*	New investigator to be added in the USA
	Except as otherwise agreed to in writing, by accepting or reviewing this document, you agree to hold this information in confidence and not copy or disclose it to others (except where required by applicable law, provided prompt written information) or use it for unauthorized purposes.	Except as otherwise agreed to in writing, by accepting or reviewing this document, you agree to hold this information in confidence and not copy or disclose it to others (*except where required by applicable law, provided prompt written notice is provided to Eloxx Pharmaceuticals and an opportunity to accord confidential treatment to the requested disclosable information*) or use it for unauthorized purposes.	Confidentiality statement updated
Signature of Investigator, 2nd paragraph 1st sentence, text added	This study will be conducted according to Good Clinical Practice (GCP) and to all stipulations, clinically and administratively, as stated in the protocol, including all statements as to confidentiality.	This study will be conducted according to Good Clinical Practice (GCP), *local regulations* and to all stipulations, clinically and administratively, as stated in the protocol, including all statements as to confidentiality.	Text added to reflect addition of new investigator in a new venue
3rd paragraph, 1st sentence, text added	It is agreed that the protocol contains all necessary information required to conduct the study as outlined in the protocol, and that the study will not be initiated without the approval of the Independent Ethics Committee (IEC) and	It is agreed that the protocol contains all necessary information required to conduct the study as outlined in the protocol, and that the study will not be initiated without the approval of the Independent Ethics Committee (IEC)*/ Institutional Review Board (IRB)* and the	Text added to reflect regulations in a new venue

Section	Used to Read	Now Reads	Rationale for Change
	the Belgian Ministry of Health/FDA.	~~Belgian Ministry of Health/FDA~~ *local health authority*.	
Additional Signature page added			Added to accommodate new investigator
PROTOCOL SUMMARY		*IND Number 137391*	IND Number added
Number of Subjects 1st sentence text added	Up to 63 healthy adult subjects will participate in the study: 9 in each cohort randomized 2:1 to ELX-02 and placebo.	Up to 63 healthy adult subjects will participate in the study *and will be recruited at multiple clinical centers*: 9 in each cohort randomized 2:1 to ELX-02 and placebo.	Clarification added due to addition of new investigator
Statistical Methods Sentence added to end of section		*Aggregate study data will be analyzed from all the cohorts, regardless of site where cohort was conducted.*	Clarification to data pooling was added
STUDY ADMINISTRATIVE STRUCTURE	Sponsor Safety Representative:	Sponsor ***Medical Director***:	Clarification of roles
CLINICAL RESEARCH ORGANIZATION	N/A	CLINICAL CENTER USA	Information to be added to reflect new investigator
5. STUDY TREATMENTS 5.1.2. Breaking the Blind 3rd paragraph, sentence added	N/A	*The Sponsor is permitted to request unblinding of any subject in association with adverse events.*	Added for clarification