STATISTICAL ANALYSIS PLAN

Protocol EL-002

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 Version and Date:	Revised Final: 29 May 2019
Name of Test Drug:	ELX-02
Developmental Phase of Study:	Phase 1b
Methodology:	Randomized, Double-Blind, Placebo-Controlled, Third Party Open, Multiple Dose Escalation
Sponsor:	Eloxx Pharmaceuticals 950 Winter Street Waltham, MA 02451 Tel: +1 (781) 577-5300
Analysis Plan Date:	25 July 2019
Analysis Plan Version:	Final Version 1.0

Confidentiality Statement

The information contained herein is confidential and the proprietary property of Eloxx Pharmaceuticals and any unauthorized use or disclosure of such information without the prior written authorization of Eloxx Pharmaceuticals is expressly prohibited.

APPROVAL SIGNATURE PAGE

Protocol 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 Number:	EL-002
Sponsor:	Eloxx Pharmaceuticals 950 Winter Street Waltham, MA 02451
Author:	[redacted]

Author Signatories:

[redacted]

Sponsor Approval:

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol, clinical development plan, and all applicable regulatory guidances and guidelines.

I have discussed any questions I have regarding the contents of this document with the biostatistical author.

I also understand that any subsequent changes to the planned statistical analyses, as described herein, may have a regulatory impact and/or result in timeline adjustments. All changes to the planned analyses will be described in the clinical study report.

[redacted]

TABLE OF CONTENTS

TABLES INCLUDED IN THE TEXT

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AE	Adverse Event
Ae	Amount excreted
Ae_{24h}	Amount excreted from time = 0 to 24 hours post-dose (also Ae_{48h}, Ae_{72h}, etc.)
AEOI	Adverse Events of Interest
ASHA	American Speech-Language-Hearing Association
AUC	Area under the concentration-time curve
AUC_t	Area under the concentration-time curve calculated to the last non-BLQ concentration
AUC_{24h}	Area under the concentration-time curve calculated to 24 hours post-dose (also AUC_{48h}, AUC_{72h}, etc.)
AUC_{inf}	Area under the concentration-time curve extrapolated to infinity
$\%AUC_{extrap}$	Percent AUC extrapolated to infinity from last observer non-BLQ concentration.
AVDSMB	Auditory and Vestibular Data Safety Monitoring Board
BLQ	Below the Lower limit of Quantification
C_{0h}	Plasma concentration at time = 0 h (predose)
C_{1h}	Plasma concentration at time 1 hour
CL/F	Apparent plasma clearance, uncorrected for fraction absorbed
CL_R	Apparent renal clearance
C_{last}	Last observed non-BLQ concentration
C_{max}	Peak plasma concentration
CPU	Clinical Pharmacological Unit
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of Variance

Abbreviation	Definition
DAIDS	Division of Acquired Immunodeficiency Syndrome
dB HL	Decibels in Hearing Level
DHI	Dizziness Handicap Inventory
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
fe	Excreted fraction (fraction of administered dose excreted)
fe_{24h}	Fraction of the dose excreted from time = 0 to 24 hours postdose (also fe48h, fe72h, etc.)
HFA	High Frequency Audiometry
ISR	Injection Site Reaction
KIM-1	Kidney Injury Molecule-1
λ_z	Terminal elimination rate constant
MedDRA	Medical Dictionary for Regulatory Affairs
MTD	Maximum Tolerated Dose
PK	Pharmacokinetic
PT	Preferred Term
PTA	Pure Tone Audiometry
$Rac_{(AUC)}$	Accumulation ratio (by AUC)
$Rac_{(Cmax)}$	Accumulation ratio (by C_{max})
R_{max}	Maximum amount excreted in a collection interval
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

Abbreviation	Definition
SC	Subcutaneous
SD	Standard Deviation
SOC	System Organ Class
SRT	Speech Reception Threshold
TEAE	Treatment-Emergent Adverse Event
THI	Tinnitus Handicap Inventory
TLF	Tables, Listings, and Figures
t_{max}	Time at which C_{max} occurs
$t_{½}$	Elimination half-life
t_{last}	Time from dose when C_{last} occurs
t_{Rmax}	Time when maximum amount is excreted
UDS	Urine Drug Screen
V_d/F	Apparent volume of distribution, uncorrected for fraction absorbed
WHO	World Health Organization

1. INFORMATION FROM THE STUDY PROTOCOL

1.1. Introduction and Objectives

1.1.1. Introduction

ELX-02, characterized as a eukaryotic ribosomal selective glycoside (ERSG), is a small molecule, new chemical entity being developed by Eloxx Pharmaceuticals for the treatment of genetic diseases caused by nonsense mutations. Nonsense mutations generating premature termination codons account for approximately one third of all genetic diseases. Nonsense mutations are a genetic mutation in a deoxyribonucleic acid (DNA) sequence that results in a shorter, unfinished protein product. Consequently, most nonsense mutations result in nonfunctional proteins (Mendell, 2001; Keeling, 2006). Examples of genetic diseases where nonsense mutations are part of their phenotype include Rett syndrome, Mucopolysaccharidosis type I (Hurler syndrome), Duchenne muscular dystrophy, and cystic fibrosis.

Across a series of pharmacology studies, the translational read-through capabilities of ELX-02 have been tested in in vitro cell models and in in vivo animal models. ELX-02 induced expression of functional proteins with pharmacodynamic and behavioral effects with a satisfactory window of safety in several cellular and animal models of genetic disease caused by nonsense mutations, including, cystic fibrosis (CF) and cystinosis. More information on ELX-02 can be found in the Investigator's Brochure.

Eloxx Pharmaceuticals has completed two Phase 1a, single-center, randomized, double-blinded placebo-controlled single ascending dose studies in healthy human subjects. After single subcutaneous (SC) administration, ELX-02 was generally well tolerated at doses ranging from 0.3 to 7.5 mg/kg without severe or serious drug-related adverse events (AEs). The mean terminal apparent half-life ranged between 2 and 8 hours. AUC_{inf} showed dose linearity and C_{max} approximate proportionality.

This multicenter study was initiated to assess the safety, tolerability and pharmacokinetics (PK) of ELX-02 in healthy human subjects receiving multiple escalating doses (ranging from 0.1 to 5.0 mg/kg) via SC injection(s) in a randomized, double-blinded, placebo-controlled design.

1.1.2. Study Objectives

The primary objectives of this study are:

- To assess the safety and tolerability of multiple ascending SC administered doses of ELX-02
- To study the PK of ELX-02 administered as multiple SC doses

The secondary objectives of this study are:

- To assess whether a maximum tolerated dose (MTD) is attained within the given dose range
- To assess dose proportionality of exposure parameters (C_{max} and AUC)

1.1.3. Purpose of this Document

This statistical analysis plan (SAP) is designed to outline the methods to be used in the analysis of study data in order to answer the study objective(s). Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of

the clinical study report for this study.

This SAP will also outline any differences in the currently planned analytical objectives relative to those planned in the study protocol.

1.2. Study Design

1.2.1. Synopsis of Study Design

This is a Phase 1, randomized, double-blind, placebo-controlled, third party open (i.e. pharmacist), multiple dose escalation study to evaluate the safety, tolerability and PK of SC 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 is designed to include at least 5 cohorts of 9 subjects each, including both males and females. Subjects will be randomized to receive multiple doses of ELX-02 or placebo at a ratio of 2:1 in each cohort; 6 subjects will receive ELX-02 and 3 will receive placebo for a total of up to 63 subjects. Study drug will be administered SC twice a week for 9 doses and dose levels planned in the protocol for each cohort are described below:

- Cohort 1: ELX-02 0.1 mg/kg or placebo
- Cohort 2: ELX-02 0.3 mg/kg or placebo
- Cohort 3: ELX-02 1.0 mg/kg or placebo
- Cohort 4: ELX-02 2.5 mg/kg or placebo
- Cohort 5: ELX-02 up to 2.5 mg/kg or placebo
- Cohort 6: ELX-02 2.5 to 5.0 mg/kg or placebo
- Cohort 7: ELX-02 up to 5.0 mg/kg or placebo

Dose escalation to the next cohort will not be performed until 14 days after the last subject's last dose in the previous cohort, to allow for detection of unanticipated delayed AEs.

The decision to proceed to a higher dose level will be following the recommendations of both the general Data Safety Monitoring Board (DSMB) and the Auditory and Vestibular Data Safety Monitoring Board (AVDSMB) based on the dose-escalation stopping rules. The DSMB and AVDSMB recommendation to proceed to a higher dose will be based on review of the blinded safety data, any available blinded PK data, and the results of audiologic tests and questionnaires. The next cohort will be dosed after the DSMB and AVDSMB have evaluated the available data and have recommended to escalate.

The principles of dose escalation, determination of dose limiting toxicity (DLT) and MTD, are detailed in Protocol Section 3.2.

Study duration will be approximately 12 weeks for each subject including a screening period of up to 6 weeks, treatment period of 4 weeks, and 2 weeks for final safety follow-up. Subjects will receive in-patient treatment in the Clinical Pharmacological Unit (CPU) for approximately 8 days throughout the treatment period.

At the time this SAP was written, five doses have been investigated and two of the dose levels involved two different dilution schemes (different concentrations being injected), for a total of

seven cohorts. The final doses (ELX-02 injection solutions) administered in the study are summarized below:

- Cohort 1: ELX-02 0.1 mg/kg (50 mg/mL) or placebo
- Cohort 2: ELX-02 0.3 mg/kg (50 mg/mL) or placebo
- Cohort 3: ELX-02 1.0 mg/kg (100 mg/mL) or placebo
- Cohort 4: ELX-02 2.5 mg/kg (100 mg/mL) or placebo
- Cohort 5: ELX-02 1.0 mg/kg (50 mg/mL) or placebo
- Cohort 6: ELX-02 2.5 mg/kg (50 mg/mL) or placebo
- Cohort 7: ELX-02 5.0 mg/kg (50 mg/mL) or placebo.

1.2.2. Randomization Methodology

Subjects who complete the study screening assessments and meet all eligibility criteria will be randomly assigned to receive either ELX-02 or placebo in a 2:1 ratio within each cohort. Within each cohort, up to 6 subjects will be randomized to receive ELX-02 and up to 3 subjects will be randomized to receive placebo for a total of up to 63 subjects. Treatment arm codes will be generated using a permuted block design. Each block will assign a 2:1 random allocation of ELX-02 or placebo using SAS® software. Each eligible subject will be assigned a unique on-study identification number. Two separate randomization schedules were created, one by SGS for Cohorts 1-6 and the other by Parexel for Cohort 7, as two separate EDC databases were created and used by these two vendors.

1.2.3. Removal, Replacement or Early Withdrawal

The Sponsor reserves the right to prematurely discontinue the study at any time for any reason. 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:

- Request by regulatory agency, Sponsor, primary care physician or Investigator
- Subject withdraws consent
- Female subject is pregnant
- Positive Urine Drug Screen (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, their continued participation will be re-evaluated on a case by case basis
- AE or Serious Adverse Event (SAE) meeting criteria for drug-related event and meeting stopping criteria described in Protocol Section 3.2.
- Subject is unwilling or unable to continue the study or is lost-to-follow-up
- Subject is non-compliant with study procedures/study protocol
- Investigator decides that withdrawal from the study is in the best interest of the subject
- Subject needs medication not allowed in the protocol

- Any clinically significant change in subject's medical condition
- Subjects may be replaced at the discretion of the Sponsor

1.2.4. Breaking the Blind

Breaking the blind is expressly forbidden except in the event of a medical emergency where the identity of the study drug must be known in order to properly treat the subject. If breaking the blind is required because of a medical emergency, the treatment identity for the unblinded subject will only be revealed by the qualified designee with approval from the Sponsor's Medical Monitor or designee. 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.

In the absence of a medical emergency, the blinded randomization for this study will not be revealed until all data are entered into the database, edits checks are performed, queries closed, electronic case report forms (eCRFs) signed by the principal investigator, and the database is officially locked.

The bioanalytical scientists and pharmacokineticists will be unblinded to treatment arm prior to database lock by having access to individual subject ELX-02 concentration data. The blind will be maintained to the clinical study team by transmitting only blinded PK summary tables and figures to the DSMB and the AVDSMB, and therefore there is no possibility of revealing individual subject treatment assignment.

1.2.5. Study Procedures

The schedule of assessments, as outlined in the study protocol, is provided in Table 1.

Table 1 Schedule of Assessments

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									X
	30 min							X														X	0-12 h								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						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
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 22						X[c]		X	X								X				X	X		X	**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 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 30	24h							X		X^q	X						X					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
	36h																					X	24-48h	X			X				X
Day 31	48h							X	X	Xq	X						X				X	X		X		X	X		X		X
Day 32	72h																					X	48-72h	X			X				
Day 36								X	X	Xq	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.

1.2.6. Efficacy, Pharmacokinetic, and Safety Parameters

1.2.6.1. Efficacy Parameters

This study does not assess efficacy.

1.2.6.2. Pharmacokinetic Endpoints and Parameters

The primary PK endpoint is to characterize plasma and urine PK of ELX-02.

The secondary PK endpoint is to determine the dose proportionality of plasma ELX-02 exposure across the administered SC dose range.

The PK parameters that will be calculated are provided in Section 4.3.

1.2.6.3. Safety Endpoints and Parameters

The primary safety endpoint is to characterize the incidence of AEs and other changes in safety parameters at ascending doses.

The secondary safety endpoint is to assess, based on the safety profile, whether dose-limiting toxicity (DLT) and MTD are attained within the tested ELX-02 dose range.

Safety evaluations performed during the study include auditory and vestibular assessments, questionnaires, ear, nose and throat physical examinations, measurement of vital signs, 12-lead electrocardiograms (ECGs), clinical laboratory evaluations (hematology, serum chemistry, urinalysis, and coagulation), and monitoring of AEs (including adverse events of interest (AEOIs), injection site reactions (ISRs), and treatment discontinuations due to AE), DLTs, and concomitant medications.

2. SUBJECT POPULATION

2.1. Population Definitions

The following subject populations will be evaluated and used for presentation and analysis of the data:

- Pharmacokinetic (PK) Population: All subjects who are randomized, receive any amount of study drug, have at least 1 evaluable PK assessment, and have no relevant deviation interfering with the PK evaluations.
- Safety Population: All subjects who are randomized and receive any amount of study drug.

The PK population is the primary population for the analysis of PK parameters. The safety population is the primary population for the analysis of safety parameters.

2.2. Protocol Deviations

Protocol deviations will be presented in a data listing.

3. GENERAL STATISTICAL METHODS

3.1. Sample Size Justification

No formal sample size estimations were performed. The planned sample size of 9 subjects per dose group with a total of up to 63 subjects was considered sufficient to address the safety, tolerability and PK objectives of this study.

3.2. General Methods

All data listings that contain an evaluation date will contain a relative study day (Rel Day). Pre-treatment and on-treatment study days are numbered relative to the day of the first dose of study drug which is designated as Day 1. The preceding day is Day -1, the day before that is Day -2, etc.

All output will be incorporated into Microsoft Word RTF or Adobe Acrobat PDF files, sorted and labeled according to the International Council on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate demographic, baseline, PK, and safety parameters. For categorical variables, summary tabulations of the number and percentage of subjects within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the number of subjects, mean, median, standard deviation (SD), minimum, and maximum values will be presented.

Summary tabulations for general and safety analyses will be presented by treatment and dose (including placebo, all dose levels and all ELX-02) and separately by concentration (including Cohorts 3, 4, 5 and 6). Placebo will be pooled across cohorts and presented in one column. A total column for those receiving ELX-02, regardless of dose, will also be included. The placebo and the total columns will not be included in the summary tabulations for PK analyses.

Figures for PK concentrations will be presented by cohort and figures for PK parameters will be presented by dose. If differences in PK parameters (AUC, C_{max}, $t_{1/2}$) between final diluted injection concentrations are observed in the "by concentration" summary tables, figures for PK parameters will be presented by cohort. Details for figure presentation are described in Section 4.3.3.

All data listings will be presented by cohort.

3.3. Computing Environment

All statistical analyses will be performed using SAS version 9.4, unless otherwise noted. PK parameters will be derived using Phoenix WinNonlin version 8.0 or higher. Medical history and AEs will be coded using Medical Dictionary for Regulatory Affairs (MedDRA) version 20.1. Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary version 20170901 (Enhanced) Format B3.

3.4. Baseline Definitions

For all analyses, baseline will be defined as the most recent measurement prior to the first administration of study drug.

3.5. Methods of Pooling Data

Data will be pooled across study sites within each treatment group (ELX-02 or placebo). Data will be pooled across cohorts for the placebo group.

3.6. Adjustments for Covariates

No formal statistical analyses that adjust for possible covariate effects are planned.

3.7. Multiple Comparisons/Multiplicity

Analyses will not be adjusted for multiple comparisons for this early phase study in healthy subjects with a primary objective of assessing safety, tolerability, and PK.

3.8. Subpopulations

No analyses of subject subgroups are planned.

3.9. Withdrawals, Dropouts, Lost to Follow-up

Subjects discontinued or withdrawn from the study after receiving ≤6 doses may be replaced upon request from the Sponsor.

3.10. Missing, Unused, and Spurious Data

In general, there will be no substitutions made to accommodate missing data points, other than described in this section. All data recorded on the eCRF will be included in data listings.

When tabulating AE data, partial dates will be handled as follows. If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as study treatment. In this case, in order to conservatively report the event as treatment-emergent, the onset date will be assumed to be the date of first dose. If the onset day and month are both missing, the day and month will be assumed to be January 1, unless the event occurred in the same year as the study treatment. In this case, the event onset will be coded to the date of first dose in order to conservatively report the event as treatment-emergent. A missing onset date will be coded as the date of first dose. If the resulting onset date is after a reported date of resolution, the onset date will be set equal to the date of resolution. Imputation of partial dates is used only to determine whether an event is treatment-emergent; data listings will present the partial date as recorded in the eCRF.

Missing AE severities will not be imputed and will be considered missing in any tabulations of AE severity. When relation of AE to the study drug is missing, the AE will be considered "related" to study drug.

When tabulating concomitant medications, if an end date is missing or the medication is ongoing at the time of first dose, the medication will be considered concomitant.

All final PK parameters will be calculated based on actual time from dose. Interim PK parameters may be calculated based upon scheduled time if actual time data are not yet available.

3.11. Visit Windows

It is expected that all visits should occur according to the protocol schedule. All data will be tabulated per the nominal visit as recorded in the eCRF even if the assessment is outside of the visit window. In data listings, the relative day of all dates will be presented (see Section 3.2).

3.12. Interim Analyses

No formal interim analyses are planned for this study. Interim blinded PK analyses are planned after each cohort from Cohort 1 through Cohort 6. Safety data will be assessed by the DSMB and AVDSMB in a blinded manner at the end of each cohort up to Cohort 6.

4. STUDY ANALYSES

4.1. Subject Disposition

Subject disposition will be tabulated and will include the number of subjects randomized to each treatment (ELX-02 or placebo), the number dosed with each treatment, and the number in each analysis population by treatment and dose and separately by concentration. The frequency and percentage of subjects who prematurely discontinue from the study, along with the primary reason for discontinuation, will also be summarized.

A by-subject data listing of study completion information including the primary reason for premature discontinuation will be presented by cohort.

4.2. Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized and presented by treatment and dose and separately by concentration. The number and percentage of subjects in each race and sex will be tabulated. Age, baseline height, baseline weight, and body mass index (BMI) will be summarized using descriptive statistics (number of subjects, mean, SD, median, minimum, and maximum). Medical history will also be tabulated.

Demographic and baseline characteristics including alcohol and stimulating beverage consumption, tobacco use, UDS, and serology (HIV, HBsAg, HCVAb) for each subject will be provided in data listings by cohort.

4.3. Pharmacokinetic Evaluations

Cohorts 1-6 blinded interim PK analyses for dose escalation recommendations by the DSMB and the AVDSMB will be the responsibility of SGS. Derivation of PK parameters for Cohorts 1-7 final analyses will be the responsibility of Early Phase QCD Team, Parexel International. For Cohorts 1-6 blinded interim analysis, the clinical team will remain blinded in that only summary tables and figures generated by the SGS team will be shared with the DSMB and the AVDSMB and no subject level data will be shared with the study team. Pharmacokinetic parameters will be calculated by noncompartmental analysis methods from the concentration-time data using Phoenix® *WinNonlin*® version 8.0 or higher.

Generation of PK tables, listings, and figures (TLFs) for the final clinical report will be the responsibility of Early Phase Biostatistics team, Parexel International.

Pharmacokinetic parameters will be generated using the PK population.

4.3.1. PK Concentrations and Parameters

4.3.1.1. Plasma PK Concentrations

Blood samples to determine ELX-02 concentrations in plasma will be drawn on Day 1 and Day 29 at the following nominal time points:

- 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).

and on Days 4, 8, 11, 15, 18 and 22 at the following nominal timepoints:

- Predose and 1h postdose (± 5 min).

4.3.1.2. Plasma PK Parameters

All final PK parameters will be calculated based on actual time from dose. Interim PK

parameters may be calculated based upon scheduled time if actual time data are not yet available. All values below the lower limit of quantification (BLQ) predose and in the absorption phase prior to the first quantifiable concentration will be substituted by zeros. All BLQ values occurring after the first quantifiable concentration will be treated as missing when calculating PK parameters.

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

- C_{max}: Maximum plasma concentration directly obtained from the experimental data of plasma concentration vs time curves, without interpolation (Day 1 and Day 29).
- t_{max}: Time corresponding to maximum plasma concentration, directly obtained from the experimental data of plasma concentration vs time curves, without interpolation (Day 1 and Day 29).
- 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 (Day 1 and Day 29).
- AUC_{24h}: Area under the plasma concentration-time curve calculated from time of administration to time 24h, computed using the linear trapezoidal rule (Day 1 and Day 29).
- AUC_{48h}: Area under the plasma concentration-time curve calculated from time of administration to time 48h computed using the linear trapezoidal rule (Day 1 and Day 29).
- AUC_{72h}: Area under the plasma concentration-time curve calculated from time of administration to time 72h, computed using the linear trapezoidal rule (Day 1 and Day 29).
- $\%AUC_{extrap}$: Percent AUC extrapolated to infinity from last observed non-BLQ concentration.
- AUC_{inf}: The area under the plasma concentration-time curve extrapolated to infinity, calculated as $AUC_t + C_{last} / \lambda_z$, where C_{last} is the last concentration above the lower limit of quantification (LLOQ) (Day 1 and Day 29). If $\%AUC_{extrap}$ is greater than 20%, the AUC value will be flagged in TLFs, footnoted, and not included in the calculation of summary statistics.
- C_{0h}: Plasma concentration observed at predose at each dosing
- C_{1h}: Plasma concentration observed at 1h postdose at each dosing.
- C_{last}: Last observed non-BLQ concentration.
- T_{last}: Time from dose when C_{last} occurs.
- $t_{½}$: Apparent elimination half-life associated with the terminal rate constant (λ_z), calculated as ln_2/λ_z (Day 1 and Day 29).
- λ_z: Terminal elimination rate constant is calculated using clearance (CL/F) and volume of distribution (V_d/F) ($\lambda_z = (CL/F)/V$).
- V_d/F: Apparent volume of distribution (Day 1 and Day 29). V_d/F (or V_z) will be calculated based on terminal phase using the equation "$Dose/\lambda_z \bullet AUC_{inf}$", uncorrected

for fraction absorbed.

- CL/F: Apparent plasma clearance, uncorrected for fraction absorbed (Day 1 and Day 29).
- $Rac_{(AUC)}$: Accumulation ratio, calculated as AUC_{72h} Day29/AUC_{72h} Day 1.
- $Rac_{(Cmax)}$: Accumulation ratio, calculated as C_{max} Day29/C_{max} Day 1.

Additional pharmacokinetic parameters may be calculated as appropriate.

4.3.1.3. Urine PK Concentrations

Urine samples to determine ELX-02 concentrations in urine will be collected at the following intervals on Day 1 and Day 29:

- Predose and during the following post-dose intervals:
  - hourly during 0-12h (± 15 min) time period, (0-1h, 1-2h, 2-3h, 3-4h, 4-5h, 5-6h, 6-7h, 7-8h, 8-9h, 9-10h, 10-11h, and 11-12h)
  - 6-hourly pooled in 12-24h (± 15 min) time period, (12-18h and 18-24h)
  - 12-hourly pooled after 24h up to 72h, (24-36h, 36-48h, 48-60h and 48-72h).
- Urine will be pooled in each interval (1, 6 or 12-hour interval), volume measured, and an aliquot will be processed to determine ELX-02 concentration.

4.3.1.4. Urine PK Parameters

The following urinary PK parameters will be calculated:

- Ae: Amount excreted in urine in an interval: 0-1h, 1-2h, 2-3h, 3-4h, 4-5h, 5-6h, 6-7h, 7-8h, 8-9h, 9-10h, 10-11h, 11-12h, 12-18h, 18-24h, 24-36h, 36-48h, 48-60h, and 60-72h on Day 1 and Day 29. Cumulative amount excreted will also be calculated, i.e. Ae_{12h}, Ae_{24h}, Ae_{48h}, Ae_{72h} .
- R_{max}: Maximum amount excreted in urine in a collection interval.
- t_{Rmax}: Time when maximum amount is excreted in urine. This will be mid-point of the collection interval.
- Fe: Percent of dose excreted in urine on Day 1 and Day 29 ($Ae_{(t)}$/dose*100), where t = 12, 24, 48, 72 (will be shown as Fe_{12h}, Fe_{24h}, Fe_{48h}, Fe_{72h}).
- CL_R: Renal clearance on Day 1 and Day 29 (CL_R = Ae_{72h} / plasma AUC_{72h}). If Ae_{72h} or AUC_{72h} is not available, CL_R will be calculated based on data up to 48 hours.

4.3.2. Dose Proportionality Assessment

Differences in PK profiles between final diluted injection concentrations (50 vs 100 mg/mL) will be explored using the figures (Section 4.3.3) and the "by concentration" tables. AUC and C_{max} will be evaluated using the ratio with its 90% confidence interval to compare Cohort 3 vs 5 and Cohort 4 vs 6. The elimination half-life, $t_{1/2}$, will be evaluated using the Kruskal-Wallis test. If no apparent differences in the PK profiles and PK parameters between the injection concentrations are observed, the PK data from two different cohorts with the same dose will be pooled together for assessing dose proportionality. Otherwise, dose proportionality will be assessed using the data from Cohorts 1, 2, 5, 6, and 7 in which the lower diluted injection concentration (50 mg/mL) was administered.

Dose proportionality will be assessed using a power model separately on Day 1 and Day 29, including the log-transformed PK parameters (C_{max}, AUC_t, and AUC_{inf}) as the dependent variables and the log-transformed dose as the fixed effect. Individual PK values will be used to perform a least-squares linear regression analysis, using the formula:

$log_{pkvar} = A + ß \times log_{dose}$, where:

- 'log_{pkvar}' represents the natural log transformed C_{max}, AUC_t, and AUC_{inf}.
- 'log_{dose}' represents the natural log transformed dose.

The slope for log-transformed dose (ß) will be estimated with its 90% confidence interval to examine dose proportionality. The PROC REG procedure will be used in SAS in the following data step:

PROC REG DATA=pkparam alpha=0.10; MODEL log_pkvar = log_dose / CLB; RUN;

The t_{max} will be compared between dose groups and days (Days 1 and 29) using the Kruskal-Wallis one-way analysis of variance (ANOVA). P-values based on the Kruskal-Wallis test will be presented.

Additional statistical analysis may be performed. If applicable, steady state will be informally assessed via a visual inspection of the figures of predose and 1-hour postdose plasma concentrations vs time (day).

4.3.3. Pharmacokinetic Data Reporting

Tables:

All plasma and urinary PK concentrations will be summarized by dose, final diluted injection concentration, day, and timepoint using descriptive statistics (number of subjects, mean, SD, median, coefficient of variance [CV%], minimum and maximum). All PK concentration and descriptive statistic values will be reported to 3 decimal places.

All plasma and urinary PK parameters will be summarized by dose, final diluted injection concentration, and day using descriptive statistics (number of subjects, mean, SD, median, CV%, minimum and maximum, geometric mean, and geometric CV%). All PK parameters and descriptive statistic values will be reported to 3 decimal places, with the exception of the individual t_{max} values, which will be reported to 2 decimal places. Footnotes will include information on parameters not included in summaries and the reasons for not including.

All BLQ, zero, and missing values of PK parameters will not be included in concentration and parameters summary calculations. If the number of non-BLQ, non-zero, and non-missing values are less than 3 in a summarization group, summary statistics will not be calculated.

Listings:

Individual plasma PK sample collection times will be listed by cohort, and will include details about subject number, cohort, dose, day, nominal sample time point, actual sample time point and concentration in the PK population. If any subjects are excluded from the PK population, a separate listing will be produced for these subjects if PK parameters are calculable.

Individual urine PK sample collection times will include details about subject number, cohort, dose, scheduled urine collection interval, actual start and end time of urine collection interval, urine volume collected in the urine collection interval, and concentration in the PK population.

All values will be reported to 3 decimal places, with the exception of the individual t_{max} values,

which will be reported to 2 decimal places.

All BLQ concentrations or missing data will be labeled as such in the concentration data listings.

Figures:

Plasma PK concentration and PK parameter figures:

- Subject profile for plasma concentration-time will be presented graphically on a linear and semi-log scale for the PK population (overlaid Day 1 and Day 29, including C_{0h} and C_{1h} on each scheduled day between Day 1 and Day 29).
- Grouped individual plasma concentration versus nominal time will be presented (Spaghetti Plots) on the linear and semi-log for the PK population (by cohort, separate Day 1 and Day 29 plots).
- Arithmetic mean (±SD) plasma concentration vs nominal time profiles will be presented graphically on a linear and semi-log scale (no SD for semi-log plot) for the PK population (by cohort, separate Day 1 and Day 29 plots, all cohorts on one plot).
- Grouped individual predose and 1h postdose plasma concentration vs nominal time from Day 1 through Day 29 will be presented (Spaghetti Plots) on the linear and semi-log scale for the PK population (by cohort).
- Mean (±SD) predose and 1h postdose plasma concentration vs nominal time from Day 1 through Day 29 will be presented on the linear and semi-log scale (no SD for semi-log plot) for the PK population (by cohort).
- Additional PK plots (by dose): Day 1 and Day 29 where applicable C_{max}, t_{max}, $t_{½}$, AUC_{24h}, Cl/F, V_d/F vs dose.

Urine PK parameter figures:

- Subject profile for amount excreted over time will be presented graphically on a linear and semi-log scale for the PK population (overlaid Day 1 and Day 29). A dashed line representing individual subject dose will be included on each plot.
- Grouped individual amount excreted versus nominal time will be presented (Spaghetti Plots) on the linear and semi-log for the PK population (by cohort, separate Day 1 and Day 29 plots).
- Arithmetic mean (±SD) amount excreted vs nominal time profiles will be presented graphically on a linear and semi-log scale (no SD for semi-log plot) for the PK population (by cohort, separate Day 1 and Day 29 plots, all cohorts on one plot).
- Additional urine PK plots (by dose), including but not limited to plasma CL/F vs CL_R, may be included as appropriate.

Details of any deviations from the planned analysis will be documented in the PK report.

4.4. Safety Analyses

All safety tabulations will be presented by treatment and dose and separately by concentration for all subjects in the Safety population.

If warranted, additional analyses of safety versus PK exposure (eg, AUC and Cmax) may be explored.

4.4.1. Study Drug Exposure

Study drug exposure will be calculated as the number of days subjects were administered study drug, as determined below, using descriptive statistics.

$$Duration\ of\ Study\ Drug\ Exposure\ (days) = (Date\ of\ last\ dose - Date\ of\ first\ dose) + 1$$

Dose intensity will be computed using the following definition and summarized by treatment and dose and separately by concentration:

$$Dose\ Intensity = \frac{Cumulative\ Actual\ Dose}{Actual\ Treatment\ Duration}$$

Relative dose intensity will be computed using the following definition and summarized by treatment and dose and separately by concentration:

$$Relative\ Dose\ Intensity\ (\%) = \frac{(Cumulative\ Actual\ Dose/Actual\ Treatment\ Duration)}{(Cumulative\ Planned\ Dose/Planned\ Treatment\ Duration)} * 100$$

Percent compliance will be summarized for each subject as:

$$Percent\ Compliance\ (\%) = \frac{Number\ of\ Injections\ Received}{Number\ of\ Injections\ Expected} * 100$$

The number of injections expected is the number defined in the protocol (9 total injections per subject). The number of expected injections for any subject who withdraws or dies prior to completing all scheduled doses will be counted up to the date of withdrawal or death.

Duration of study drug exposure, number of doses received, dose intensity, relative dose intensity, and percent compliance for each subject will be listed by cohort.

4.4.2. Adverse Events

All AEs will be coded using the MedDRA coding system and displayed in tables and data listings using system organ class (SOC) and preferred term (PT). AEs will be presented by severity (mild, moderate, or severe).

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE occurring after the first dose of study medication. Adverse events reported prior to dosing will be considered non treatment-emergent.

The number and percentage of subjects as well as the number of events for each particular treatment-emergent AE (TEAE) will be summarized by treatment and dose, concentration, SOC, and PT for the following: TEAEs, TEAEs assessed by the Investigator as related to treatment, severe TEAEs, serious TEAEs, TEAEs leading to permanent study drug discontinuation, TEAEs leading to temporary discontinuation, TEAEs leading to dose reduction, TEAEs by maximum severity, and TEAEs by relationship to study drug.

In these tabulations for the number and percentage of subjects, each subject will contribute only once (i.e., the most related occurrence or the most intense occurrence) to each of the incidence rates for a given SOC or PT in the descriptive analysis, regardless of the number of episodes.

For tabulations that include classification by relationship to study treatment, AEs with missing relationship will be considered related to study drug. For tabulations that include classification

by severity, AEs with missing severity will be included in a category for missing.

All AEs occurring on-study will be listed in by-subject data listings by cohort.

By-subject data listings will also be provided for the following: subject deaths, SAEs, severe TEAEs, TEAEs leading to treatment discontinuation, and TEAEs leading to dose reduction.

4.4.3. Adverse Events of Interest and Injection Site Reactions

Treatment-emergent AEOIs, defined per-protocol as hypersensitivity, nephrotoxicity, and ototoxicity, will be assessed. AEOIs, searched by the following standardized MedDRA queries (SMQs), will be summarized by PT: Hypersensitivity, Hearing and Vestibular Disorders, and Acute Renal Failure, as appropriate. The number and percentage of subjects with at least one AEOI as well as the number of AEOIs will be presented by treatment and dose and separately by concentration.

ISRs, defined per-protocol as pain or tenderness, erythema or redness, induration or swelling, and pruritus, and assessed by the investigators according to the Division of Acquired Immunodeficiency Syndrome (DAIDS) Criteria as described in Protocol Section 13.1 will be summarized by grade at each timepoint.

ISRs reported as spontaneous AEs that are coded with the high-level term (HLT) "Injection site reactions" will also be analyzed. The number and percentage of subjects with at least one ISR as well as the number of ISR events will be presented by treatment and dose and separately by concentration. The duration of each reported ISR will be derived using the AE dataset. The average duration of ISRs per subject will be summarized by treatment and dose and separately by concentration.

All AEOIs and ISRs occurring on-study as well as the duration of each observed ISR will be listed in by-subject data listings by cohort.

4.4.4. Laboratory Data

Clinical laboratory values will be expressed in Système International (SI) units.

The actual value and change from baseline (Day 1) to each on-study evaluation will be summarized for each clinical laboratory parameter, including hematology, clinical chemistry, urinalysis, and coagulation and presented by treatment and dose and separately by concentration. In the event of repeat values, the last non-missing value per study day/time will be used. Laboratory results recorded at unscheduled visits will not be tabulated.

Severity of clinical laboratory measurements will be determined using Common Terminology Criteria for Adverse Events (CTCAE) criteria when available.

Shift tables of change in CTCAE grade of laboratory parameters from baseline to worst value and from baseline to last value on study will be presented. Both scheduled and unscheduled visits will be included in shift tables.

All laboratory data will be provided in by-subject data listings, including results of pregnancy tests, alcohol tests, and serology. A subset listing will be presented for all laboratory values with CTCAE grade $\geq$3.

4.4.5. Vital Signs and Physical Examination

The actual value and change from baseline to each on-study evaluation will be summarized for

vital signs, including blood pressure (systolic/diastolic), heart rate, body temperature, and respiratory rate and presented by treatment and dose and separately by concentration.

Vital sign measurements and all physical examination findings will be presented in by-subject data listings by cohort.

4.4.6. Auditory and Vestibular Assessments

Audiometric and vestibular tests will be performed at Screening, Baseline, Day 3, Day 10, Day 17, Day 24 and end of study (EOS).

4.4.6.1. Otoscopy

The number and percentage of subjects with otoscopic examination results (normal, abnormal, not done) for each ear will be summarized by visit, treatment and dose, and separately by concentration.

Otoscopic examination results for each ear and comments will be listed by cohort, subject and visit.

Use of hearing aid and regular/history of excessive noise exposure will be provided in by-subject data listings.

4.4.6.2. Pure Tone Audiometry and High Frequency Audiometry

Air conduction pure tone audiometry (PTA) will be conducted across the conventional range of frequencies (0.25, 0.5, 1, 2, 3, 4, 6, 8 kHz), as well as at high frequencies (10, 12, 14, 16 kHz). If there is a PTA threshold ≥15 decibels in hearing level (dB HL) for frequencies between 0.25 - 4 kHz, bone conduction will be performed at that frequency.

PTA/High Frequency Audiometry (HFA) threshold and change from baseline in PTA/HFA threshold for each ear at each frequency will be summarized descriptively (n, mean, SD, median, min, median, max) at each visit by treatment and dose, and separately by concentration.

Mean (±SD) changes from baseline in PTA/HFA threshold by dose will be plotted across all visits for each ear at each frequency.

For conventional frequencies (0.25 - 8 kHz) and HFA (10 - 16 kHz), the Significant Change Criteria as defined by American Speech-Language-Hearing Association (ASHA) and American Academy of Audiology (AAA) are when the thresholds worsen by the following criteria:

1. ≥ 20 dB change at any frequency,
2. ≥ 10 dB change at any 2 adjacent frequencies, or
3. loss of response at 3 consecutive frequencies where responses were obtained at baseline.

The number and percentage of patients with significant change relative to baseline across the conventional frequencies (yes, no) and significant change relative to baseline at the high frequencies (yes, no) will be summarized for each ear by visit, treatment and dose, and separately by concentration.

PTA and HFA data for each subject will be listed by cohort.

4.4.6.3. Speech Reception Threshold

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.

The number and percentage of responses (0-100, >100, no response, not done) will be summarized for each ear by visit, treatment and dose, and separately by concentration.

SRT response for each ear will be provided in by-subject data listings by cohort.

4.4.6.4. Tympanometry

The number and percentage of subjects with each type (A, As, Ad, B, C, not done) will be summarized for each ear by visit, treatment and dose and separately by concentration.

Tympanogram type for each ear and any abnormal tympanogram or other indication of middle ear abnormality will be provided in by-subject data listings by cohort.

4.4.6.5. Tinnitus Handicap Inventory Questionnaire

Tinnitus Handicap Inventory (THI) total score and change from baseline in THI total score will be summarized descriptively (n, mean, SD, median, min, max) at each visit by treatment and dose, and separately by concentration.

The number and percentage of subjects who have a score of a 5-point increase or greater in THI score from baseline accompanied by a shift in Grade (eg, from Grade 1 to Grade 2) as shown in the following table will be presented by treatment and dose, and separately by concentration.

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.

Individual questions as well as total scores for the THI will be provided in by-subject data listings by cohort.

4.4.6.6. Dizziness Handicap Inventory Questionnaire

Dizziness Handicap Inventory (DHI) total score and change from baseline will be summarized descriptively (n, mean, SD, median, min, max) at each visit by treatment and dose, and separately by concentration.

The number and percentage of subjects who have a score of 31 or greater on the DHI, a 5-point increase or greater from baseline, and an associated shift to the following category (eg, from mild to moderate handicap) will be summarized by treatment and dose, and separately by concentration.

- 16-34 points (mild handicap)

- 36-52 points (moderate handicap)
- 54 + points (severe handicap)

Individual questions and total scores for the DHI will be provided in by-subject data listings by cohort.

4.4.7. Electrocardiogram

Actual values and change from baseline to each on-study evaluation will be summarized for the following ECG results, heart rate, PR, QRS, QT, QTcB, and QTcF intervals.

ECG data for each subject will be provided in by-subject data listings.

4.4.8. Renal Injury Biomarkers

Renal injury biomarkers include KIM-1 and clusterin in urine. Renal injury biomarkers will be normalized by urine creatinine and computed using the following definition:

Renal Injury Biomarker / Urine Creatinine.

Actual value and changes from baseline in each of the renal injury biomarkers normalized by urine creatinine will be summarized descriptively at each timepoint by treatment and dose, and separately by concentration.

Renal injury biomarkers, urine creatinine and normalized renal injury biomarkers for each subject at each timepoint will be provided in data listings by cohort.

4.4.9. Concomitant Medications

Concomitant medications will be coded using the WHO Drug Dictionary. Results will be tabulated by anatomic therapeutic class (ATC), PT, treatment and dose, and separately by concentration.

Concomitant medications will be tabulated, where any medications that were not discontinued prior to the first dose of study medication will be included.

The use of concomitant medications will be included in a by-subject data listing by cohort.

5. CHANGES TO PLANNED ANALYSES

The study secondary objective remains unchanged, but the wording has been clarified to be more precise from "to assess linearity between ascending SC doses and PK parameters" to "to assess dose proportionality of exposure parameters (C_{max} and AUC)". Subsequently, it is clarified furthermore in Section 4.3.2 that the dose proportionality will be assessed using a power model separately on Day 1 and Day 29, including the log-transformed PK parameters as dependent variables and the log-transformed dose as the fixed effect. The protocol specified fixed effects of day and dose*day are not required for the model that is performed separately on each individual day and therefore are excluded from the power model.

6. REFERENCES

Keeling K, Du M, Bedwell DM: Therapies of nonsense-associated diseases. In: Nonsense-Mediated mRNA Decay. edn. Edited by Maquat LE. Austin, TX: Landes Biosciences; 2006: 121-136.

Mendell JT, Dietz HC: When the message goes awry: disease producing mutations that influence mRNA content and performance. *Cell* 2001, 107(4):411-414.