A Phase 2 Open Label Pilot Study to Evaluate the Safety and Efficacy of Subcutaneously Administered ELX-02 in Patients with Alport Syndrome with Col4A5 and Col4A3/4 Nonsense Mutation

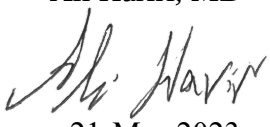
Compound:	ELX-02
Compound Name:	6'-(R)-Methyl-5-O-(5-amino-5,6-dideoxy-α-L-talofuranosyl)- paromamine sulfate
European Clinical Trials Database (EudraCT) Number:	2022-000604-35
Protocol Number:	EL-014
Phase:	2
Sponsor:	Eloxx Pharmaceuticals Inc 480 Arsenal Way, Suite 130 Watertown, MA 02472 United States

Signature of Sponsor Representative

A Phase 2 Open Label Pilot Study to Evaluate the Safety and Efficacy of Subcutaneously Administered ELX-02 in Patients with Alport Syndrome with Col4A5 and Col4A3/4 Nonsense Mutation

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

Print Name: Ali Hariri, MD

Signature: 

Date: 21-Mar-2023

Signature of Investigator

A Phase 2 Open Label Pilot Study to Evaluate the Safety and Efficacy of Subcutaneously Administered ELX-02 in Patients with Alport Syndrome with Col4A5 and Col4A3/4 Nonsense Mutation

Name:

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 participants.

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 authority.

Signature:

Date:

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PROTOCOL SYNOPSIS

Study Title	A Phase 2 Open Label Pilot Study to Evaluate the Safety and Efficacy of Subcutaneously Administered ELX-02 in Patients with Alport Syndrome with Col4A5 and Col4A3/4 Nonsense Mutation
Protocol No.	EL-014
Phase	2
Test Product, Dose, and Mode of Administration	ELX-02: 6'-(R)-methyl-5-O-(5-amino-5,6-dideoxy-α-L-talofuranosyl)- paromamine sulfate ELX-02 is a synthetic ERSG formulated as lyophilized powder for injection producing a concentration of 50 mg/mL upon reconstitution. ELX-02 will be administered at a dose of 0.75 mg/kg SC daily for 60 days.
Background and Rationale	Alport Syndrome (AS) is a progressive hereditary renal disease frequently accompanied by extrarenal manifestations including sensorineural hearing loss, cataracts and slow decline in vision. AS is the second most common renal genetic disease occurring at a rate of 1 in 2500–6000 live births. It is caused by mutations in the Col4A3, Col4A4 or Col4A5 (Col IV) genes. These type IV collagens constitute the main basement membrane in kidney and ear responsible for structural integrity of the tissue. Defects in basement membrane in kidney glomerulus results in breaks in the glomerular capsule causing glomerular injury, proteinuria, hematuria, decrease in glomerular filtration leading to fibrosis and eventual renal failure. Similarly, loss of basement membrane integrity in the inner ear leads to progressive hearing loss. AS mutations are X linked (80%) and autosomal (20%), out of which approximately 6-8% are due to nonsense mutation. No approved treatment is available for AS patients; those with nonsense mutations have the most severe disease, with early onset of disease and significant morbidity and mortality (leading to early onset ESRD and deafness). So far, the main treatment for AS is off-label use of ACEi/ARBs to delay disease progression followed by renal replacement therapy to treat renal failure. ELX-02 is an investigational eukaryotic ribosomal selective glycoside (ERSG) optimized as a translational read-through molecule that induces read-through of nonsense mutations resulting in production of full-length functional proteins demonstrated across many diseases *in vitro* and *in vivo* in animal models. ELX-02 has also been tested in Alport Syndrome nonsense mutations in vitro where it has consistently shown read-through activity resulting in structural reconstitution of Col IV 3,4,5 trimer. The safety and pharmacokinetics (PK) of ELX-02 have been tested in single and multiple dose healthy volunteer studies, renal impairment volunteers, and in Cystinosis and Cystic Fibrosis patients. Physiologic Based Pharmacokinetic (PBPK) Modeling of ELX-02 has shown >50-fold increased exposure in kidney versus plasma creating a robust potential for Collagen IV 3, 4, 5 trimer reconstitution by readthrough of the nonsense mutation. This makes it an attractive approach to personalized Alport Syndrome therapy.
Objectives	<u>Primary Objectives</u> 1. To assess the safety and tolerability of ELX-02 administered subcutaneously (SC) in patients with Alport syndrome with Col4A5 and Col4A3/4 nonsense mutations.

	Secondary Objectives 1. To assess the effect of ELX-02 on proteinuria. 2. To assess the effect of ELX-02 on expression of Col IV protein in kidney. 3. To assess the effect of ELX-02 on hematuria. Exploratory Objectives 1. To assess the effect of ELX-02 on potential improvement in hearing of the patients with compromised hearing. 2. To assess Col IV protein levels in urine and or kidney cells, if warranted.
Endpoints	Primary Endpoints: 1. The incidence and characteristics of adverse events (AEs). Other safety assessments will include injection site reactions (DAIDS Grading Scale) and changes from baseline in vital signs, physical examination, electrocardiograms, blood and urine safety laboratory tests, audiometric testing (including high frequency audiometry), Tinnitus and Dizziness status. Secondary Endpoints: 1. Change from baseline to EOT and EOS in proteinuria. 2. Change from baseline to EOT in Col IV expression in renal biopsy 3. Change from baseline to EOT and EOS in hematuria. Exploratory Endpoints: 1. Potential improvement in audiometric testing (including high frequency audiometry) in individuals with baseline abnormalities. 2. Change in Col IV protein levels in urine and/or kidney cells based on changes in urine.
Study Design	This is a Phase 2 open label pilot study to evaluate the safety and efficacy of subcutaneously administered ELX-02 in patients with X-linked or autosomal recessive Alport Syndrome with Col4A5 and Col4A3/4 nonsense mutation. In total, up to 8 participants, with a minimum of 3 adults, will be enrolled in the trial. In case of a screen failure, participants may be re-screened at the discretion of the investigator. The study will be comprised of the following periods for each participant: • a Screening period of up to 6 weeks (42 days) • a total Treatment Period of 8 weeks (60 days) • a safety/efficacy Follow-up Period of 12 weeks (90 days) after the last treatment The Treatment Period will be a treatment of ELX-02 0.75 mg/kg SC QD for 8 weeks. During the treatment period, participants will receive their first dose of study drug at the clinic and have study assessments done (Day 1). Participants will return to the clinic for a study visit every 2 weeks for safety monitoring, efficacy and return/re-dispensing of study drug ELX-02. Except on study visits, treatment will be self-administered (or by a trained care provider) at participant's home. Participants (and/or caregivers) will be instructed on self-administration to ensure safety and compliance.

	The study will initially enroll participants 12-30 years old only. Once the first two participants of ages 12-18 years complete 4 weeks of study drug treatment, a DSMB will evaluate the safety and PK data of these two sentinel participants. Following DSMB review and decision on the safety profile of the study drug, participants 6-11 years old can be dosed with the study drug. If needed, the DSMB will review reported potentially related serious adverse events (SAEs) leading to discontinuation or study stoppage as they are reported. The determination of relatedness will be made by the treating investigator (Section 8.9). At home, ELX-02 daily dose should preferably be taken at approximately the same time every day. Subcutaneous injection(s) of ELX-02 are to be administered to the abdominal area or any area with a significant fat deposit (such as the upper thigh). Caution should be taken to ensure the injection is not administered into muscle. A participant should not have elective injectable aminoglycoside during days of active ELX-02 treatment and at least 14 days before or after ELX-02 treatment. For any unplanned/urgent injectable aminoglycoside use, ELX-02 administration should be suspended and restarted 14 days after aminoglycoside treatment completion. All participants will proceed through the entire Treatment Period unless they meet an individual stopping criterion (shown below), or the study meets the stopping criteria (see Section 4.8). <u>Dose stopping in an individual participant:</u> • Injection site reactions: o The occurrence of a DAIDS grade 3 or higher injection site reaction • Nephrotoxicity as defined by Grade 2 Common Terminology Criteria for Adverse Events (CTCAE Version 5.0) based on serum creatinine • Participants who evidence a Grade 2 CTCAE hearing impairment in the conventional frequencies or high frequency threshold shift meet stopping criteria for suspected ototoxicity. For adults, this is specified as a worsening shift from baseline of >25 dB averaged at 2 contiguous test frequencies in at least one ear, or at least 5-point worsening on THI or DHI questionnaires and category change. For pediatric participants, this is specified as a worsening shift from baseline of >25 dB averaged at 2 contiguous test frequencies in at least one ear including a worsening shift of > 20 dB at 4 kHz in at least one ear. • Any other adverse event (AE), which in the opinion of investigator puts the participant at undue risk If it is determined following a thorough review that the initial assessment was incorrect, treatment of the participant may be resumed. This determination will be made by the DSMB in agreement with the Sponsor. Participants who withdraw prior to study completion for non-safety reasons (e.g., personal reasons or non ELX-02 related AEs) may be replaced upon approval of the Sponsor.
Inclusion Criteria	Participants must meet all of the following criteria to participate in this study: 1. Evidence of signed and dated informed consent/assent document(s) indicating that the participant (and/or their parent/legal guardian) has been informed of all pertinent aspects of the trial

	2. Understands, and is willing and able to comply with all scheduled visits, treatment plan, laboratory tests, and other study procedures 3. Male and female participants 4. Ages between 6 years to 30 years 5. A confirmed diagnosis of X-linked or autosomal recessive Alport Syndrome with a documented nonsense mutation of Col4A5 in a male or nonsense mutation of Col4A3 or Col4A4 (male or female) 6. The nonsense mutation should be UAG or UGA 7. eGFR≥60 ml/min/1.73 m^2 (based on CKD-EPI for ages ≥18 and Schwartz formula for participants <18) 8. Urinary protein based on two spot urine collections [urine protein/creatinine ratio (UPCR) ≥ 500 mg/g] 9. Stable regimen of ACEi/ARB for at least 4 weeks before screening (unless there is a contraindication) 10. Must be willing to abstain from strenuous exercise during the 48 hrs prior study visits 11. Females of childbearing potential and males capable of fathering a child must meet the contraception requirements outlined in Section 6.4 12. Non-lactating females 13. Females on hormone replacement therapy (estrogen or progesterone) or contraceptive therapy must be stabilized on a product and dose for at least 30 days prior to Screening 14. Have not received systemic medications with potential to impair renal function on a frequent basis (e.g., nonsteroidal anti-inflammatory drugs [NSAIDs]) or with ototoxic potential (e.g., quinine or salicylates), or any injectable aminoglycosides for a period of at least 14 days prior to dosing 15. Negative human immunodeficiency virus (HIV), hepatitis B surface antigen (HbsAg), and hepatitis C virus antigen (HCV Ag) serology tests at Screening 16. No history of alcohol or drug abuse within the 6 months prior to Screening 17. Body Mass Index (BMI) of 19.0 to 30.0 kg/m^2 (inclusive). Participants with a lower BMI may be entered into the study at the discretion of the investigator following consultation with the Sponsor
Exclusion Criteria	Participants with any of the following characteristics/conditions will not be included in the study: 1. Participants 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 participants who are Eloxx Pharmaceuticals employees directly involved in the conduct of the study 2. Participation in clinical study including administration of any investigational drug or device in the last 30 days or 5 half-lives (whichever is longer) prior to investigational product dosing in the current study 3. Use of prohibited medications as defined in Section 6.1 within the specified windows 4. History of any comorbidity which in the opinion of the investigator might confound the study or pose an additional risk in administering the study drug to the participant

	5. History of any organ transplantation 6. Mutation consistent with autosomal dominant Alport Syndrome 7. Liver disease characterized by cirrhosis or portal hypertension. Participants with alanine aminotransferase (ALT), aspartate aminotransferase (AST), and/or a total bilirubin 3.0 times the upper limit of normal (ULN) will be excluded 8. History of congestive heart failure diagnosed clinically or with documented left ventricular ejection fraction (LVEF) ≤ 40% 9. Evidence or history of clinically relevant psychiatric condition 10. A positive urine drug screen (amphetamines, benzodiazepines, cocaine and opiates) at Screening 11. Screening supine 12-lead electrocardiogram (ECG) demonstrating any clinically significant findings as judged by the Investigator 12. Participants with any abnormalities in clinical laboratory tests at Screening, considered by the study Investigator as clinically relevant 13. Major surgery within 180 days (6 months) of Screening 14. History of dialysis 15. Known allergy to any aminoglycoside 16. Participants with any acute medical situation unresolved within 14 days of first dose that is considered of significance by the Investigator 17. Participants with >10 dB change in threshold between audiometric Initial test to Baseline test for the frequencies from 0.5 -12.5 kHz 18. Dizziness Handicap Inventory (DHI) score at screening ≥16 for adults, and active dizziness reported for pediatric participants.
Statistical Methods	No formal sample size or power calculation has been performed. Safety analyses will be based on the Safety Population, defined as all treated participants who received at least one dose of study drug, including participants prematurely withdrawn from the study. PK analyses will be based on the PK Population defined as those treated participants who have analyzable PK data without relevant deviation interfering with the PK evaluations. Efficacy analyses will be based on the efficacy Population defined as those participants who have a valid baseline (before dosing) efficacy sample and at least 1 non-missing post-baseline value for an efficacy sample. Safety, PK and efficacy data will be summarized using descriptive statistics and/or graphical displays. Efficacy parameters will be described as changes from baseline in PD measurements during each treatment period. Changes in hematuria will be recorded as a categorial variable. Proteinuria will be evaluated as UPCR. The baseline value will be the geometric mean of the screening and pre-Day 1 dosing samples.

ABBREVIATIONS

Abbreviation	Term
AAA	American Academy of Audiology
AE	Adverse events
Ae	Cumulative amount of unchanged drug excreted into urine
AESI	Adverse event of special 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
BIW	Twice weekly
BMI	Body mass index
BP	Blood pressure
CF	Cystic fibrosis
CKD-EPI	Chronic kidney disease epidemiology collaboration
CL/F	Apparent plasma clearance
C_{max}	Maximum plasma concentration
CNS	Central nervous system
COVID-19	Coronavirus disease of 2019
CPR	C-reactive protein
$C_{predose}$	Trough plasma concentration observed at the end of the dosing interval (i.e., at each predose starting from second dose)
CRF	Case report form
CRO	Clinical research organization
CSA	Clinical study agreement
CTA	Clinical trial application
CTCAE	Common Terminology Criteria for Adverse Events
DAIDS	Division of Aids
DHI	Dizziness Handicap Inventory
DMD	Duchenne muscular dystrophy
DNA	Deoxyribonucleic acid

Abbreviation	Term
DOA	Drugs of abuse
DSMB	Data Safety Monitoring Board
EDC	Electronic data capture
EDP	Exposure during pregnancy
ECG	Electrocardiogram
ENT	Ear, nose, and throat
EOS	End of study
ESRD	End-Stage Renal Disease
ERSG	Eukaryotic ribosomal specific glycoside
eTMF	Electronic trial master file
EU	European Union
FDA	Food and Drug Administration
FSH	Follicle stimulating hormone
GAG	Glycosaminoglycans
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HbsAg	Hepatitis B surface antigen
HCT	Hematocrit
HCV Ab	Hepatitis C virus antibody
HCV Ag	Hepatitis C virus antigen
HED	Human equivalent dose
HFA	High frequency audiometry
HIV	Human Immunodeficiency Virus
HR	Heart rate
Hr(s)	Hour (s)
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
IMPD	Investigational Medicinal Product Dossier
IRB	Institutional Review Board
ISR	Injection Site Reaction
IV	Intravenous
K	Potassium
LBM	Lean body mass

Abbreviation	Term
LH	Luteinizing hormone
LLOQ	Lower limit of quantification
LPLV	Last visit of the last participant in the study
LS	Life sciences
LVEF	Left ventricular ejection fraction
MA	Medical Affairs
MDRD	Modification of Diet in Renal Disease
MPS	Mucopolysaccharidose type I
min	Minute
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
NSAIDs	Non-steroidal anti-inflammatory drugs
OTC	Over the counter
PBPK	Physiologically based pharmacokinetics
PD	Pharmacodynamic(s)
PI	Principal Investigator
PK	Pharmacokinetic(s)
PT	Preferred term
PTA	Pure tone audiometry
Rac	Accumulation ratio
RBC	Red blood cells
RNA	Ribonucleic acid
rRNA	Ribosome ribonucleic acid
RR	Respiratory rate
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous(ly)
SD	Standard deviation
SOC	System organ class
SRT	Speech Reception Threshold

Abbreviation	Term
SUSAR	Suspected unexpected serious adverse reaction
$t_{½}$	Elimination half-life
TEAE	Treatment-emergent adverse event
THI	Tinnitus Handicap Inventory
t_{max}	Time to maximum concentration
tRNA	Transfer ribonucleic acid
ULN	Upper limit of normal
UMC	Uppsla Monitoring Center
UPCR	Urine Protein/Creatinine Ratio
USA	United States of America
UTR	Untranslated region
Vd/F	Apparent volume of distribution
WBC	White blood cell
WHO	World Health Organization

STUDY ADMINISTRATIVE STRUCTURE

SPONSOR	Eloxx Pharmaceuticals 480 Arsenal Way, Suite 130 Watertown, MA 02472 United States
Chief Medical Officer:	Ali Hariri, MD
Senior Director, Clinical Operations:	Lior Horonchik, Ph.D.

1 INTRODUCTION AND RATIONALE

1.1 Alport Syndrome

Alport syndrome (AS) is a progressive hereditary renal disease frequently accompanied by extrarenal manifestations including sensorineural hearing loss, cataracts and slow decline in vision. AS is the second most common renal genetic disease occurring at a rate of 1 in 2500–6000 live births. It is caused by mutations in the Col4A3, Col4A4 or Col4A5 (Col IV) genes. These type IV collagens constitute the main basement membrane in kidney and ear responsible for structural integrity of the tissue. Defects in basement membrane in kidney glomerulus results in breaks in the glomerular capsule causing glomerular injury, proteinuria, hematuria, decrease in glomerular filtration leading to fibrosis and eventual renal failure. Similarly, loss of basement membrane integrity in the inner ear leads to progressive hearing loss.

Renal progression of disease goes through several stages. Microscopic hematuria is the earliest manifestation of disease. This evolves to microalbuminuria (UACR30-300 mg/g) and gross proteinuria (UACR>300 mg/g). Consequently, proteinuria reduction and improvement in hematuria is the best representation of the reversal of the disease.[1] After initial glomerular injury due to basement membrane breaks, continued cycles of injury and scarring leads to fibrosis of the kidney and eGFR decline progressing to renal failure requiring dialysis or transplantation. It is not surprising that 0.5% and 12.9% of newly developed renal failure in adults and children respectively is due to AS, while particularly, patients with AS who harbor nonsense mutations suffer from juvenile onset of ESRD.[2]

X-linked AS, is the most common inheritance pattern and is caused by mutations in Col4A5. Mutations in Col4A3 and Col4A4 result in autosomal transmission manifesting either as recessive or as dominant pattern of inheritance. The frequency of X-linked AS, autosomal recessive AS, and autosomal dominant AS are estimated to be 80%, 15% and 5% respectively.[3] AS is frequently underdiagnosed due to atypical presentation in a significant fraction of patients. Genetic analysis of patients with chronic kidney disease shows that only 38% of the patients with Col IV mutations are properly diagnosed as Alport patients.[4]

Among the different mutations in these three genes, approximately 6% are due to nonsense mutations.[5] Patients with nonsense mutations along with those having large rearrangements, frameshift or splicing abnormalities resulting in truncated or absent protein, [2, 6] present with the most severe phenotype including early onset of disease, significant morbidity and mortality leading to early onset ESRD and deafness.[2, 7, 8] The mean age of ESRD in these patients is 20 years of age. In contrast, patients with missense Col4A5 mutations (such as glycine -XY mutations) have a mean age of ESRD as 30 years of age.[2] In a more recent study,[5] it was reported that nonsense mutations in Col4A5 and Col4A3/Col4A4 have an onset of ESRD of 21.4 and 17.9 years of age respectively. At the same time the less severe mutations have the age of ESRD of 26.7 (col4A5) and 24-26.6 (Col4A3/Col4A4). In patients with Col4A5 nonsense mutations, the onset of ESRD occurred as early as 16.9 years and in patients with Col4A4/Col4A3 nonsense mutations as early as 13.3 years. The early onset and rapid progression to renal failure in AS patients with nonsense mutations makes developing therapies in this pre-adolescent and adolescent patients urgent.

Despite the high unmet need, there is no approved treatment for AS. Use of ACEi/ARBs to delay disease progression followed by renal replacement therapy to treat eventual renal failure is the main treatment approach. Recent studies have shown that early initiation of with ACEi/ARB in pediatric

age results in significant delay in renal progression.[9] Specifically, early proteinuria reduction by ramipril improved renal survival in children with AS (ESCAPE trial and PROTECT trials). While ACEi/ARBs provide a small degree of renal protection AS patients reach ESRD at a young age, there is no special treatment for hearing loss other than use of cochlear implants or hearing aids.

1.2 Nonsense Mutation Readthrough

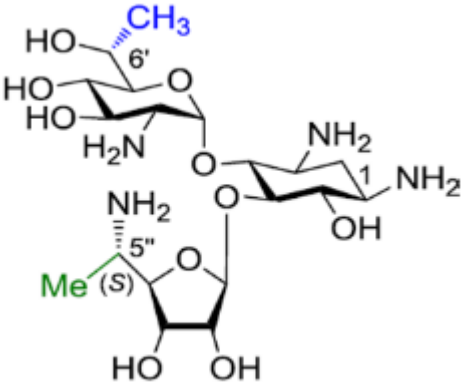
ELX-02 is an investigational eukaryotic ribosomal selective glycoside (ERSG) optimized as a translational read-through molecule that induces read-through of nonsense mutations resulting in production of full-length functional proteins demonstrated across many diseases in vitro and in vivo in animal models. Nonsense mutations are genetic mutations in a deoxyribonucleic acid (DNA) sequence that results in a shorter, truncated non-functional protein product. During protein formation, an 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 3' untranslated region (UTR), including several release factors, signal the end of protein synthesis. Thus, nonsense mutations occur when a premature stop 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.[10, 11]

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 is intended to read through the nonsense mutation and induce the translation of full-length functional proteins to have a beneficial clinical effect in these diseases. Comprehensive preclinical testing of ELX-02 in vitro and in animal models of disease demonstrated its potential efficacy and decreased toxicity compared to traditional aminoglycosides. Thus, ELX-02 has the potential to treat any disease caused by nonsense mutations and thus may have broad applicability for many orphan diseases. In addition, based on the ELX-02 PK studies have shown 50X uptake in the kidney and ear.

1.3 ELX-02

ELX-02 is a new non-antibiotic aminoglycoside analogue [6'-®-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 has poor oral bioavailability but rapid parenteral (subcutaneous [SC] and intravenous [IV]) absorption, reaching peak plasma concentrations within 30 to 60 minutes after administration.

ELX-02 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 Investigator's Brochure (IB).

The pharmacokinetic (PK) 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. The PK profile of ELX-02 in animal tissues is complex. Like in plasma, ELX-02 follows a multi-phasic decay with a large elimination phase that accounts for 61 to 95% of the AUC and a low distribution phase that accounts for 5 to 39% of the AUC after weekly or biweekly dose for 28-days. The kidneys account for the majority (68 to 75%) of the area under the plasma concentration-time curve (AUC) in the distribution phase. In some tissues, the half-life of ELX-02 is longer than the dosing interval so that, with multiple dosing, ELX-02 accumulates. Tissue half-life of ELX-02 ranges from 3.5 to 116 hrs. Extensive physiologically based pharmacokinetic (PBPK) modeling in animals and humans allows predictions of time-dependent ELX-02 tissue concentrations, given a dose and dosing interval so that, for example, measurement of plasma and urine ELX-02 levels inform on renal distribution.

ELX-02 targets peripheral pharmacological compartments where it accumulates to varying degree and with compartment-specific PK. The therapeutic value of ELX-02 is a direct consequence of specific targeting of peripheral pharmacological compartments such as kidney, lung, muscle, and CNS with a controlled exposure sufficient to promote expression of functional protein that, in turn, leads to efficacious outcomes below toxic exposures, particularly in the kidney and ear. In this manner, ELX-02 is expected to induce controlled translational read-through of premature termination codons in each tissue for a mutated gene specific for a given genetic disease.

1.4 Mechanism of Action – The Effect of Aminoglycosides and ELX-02 on Nonsense Mutations

ELX-02 is a rationally drug candidate designed to induce readthrough across the premature termination codon (also known as nonsense mutation) resulting in full-length protein production and restoration of protein function. The basis for rational design of ELX-02 is established readthrough activity noted for aminoglycoside antibiotics like gentamicin.

Aminoglycosides bind to the decoding site in the small subunit of the ribosome ribonucleic acid (rRNA)[12] that normally monitors proper codon-anticodon interactions and is capable of inducing 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[13] and stabilizes 3 critical adenosine residues in a conformation suitable for read-through[14]. This reduction in the accuracy of codon-anticodon recognition increases the probability that translational read-through of stop codons occurs. Despite promising results, aminoglycoside use as a long-term translational read-through 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.[15,18,19]

The readthrough activity of ELX-02 was optimized through successive rounds of medicinal chemistry that took into account the known structure activity relationship of aminoglycosides [20] [21,22,23,14]

Rational design separated the structural elements of the aminoglycoside scaffold inducing read-through from those elements affecting antibacterial activity.[24,25] This led to preferential binding of

ELX-02 to the eukaryotic ribosome with no antibiotic activity. Low affinity of ELX-02 for the prokaryotic ribosome decouples the antibacterial activity from read-through activity. When compared to gentamicin, ELX-02 demonstrates a 100-fold lower antibacterial activity and 9-fold higher read-through activity, which is attributed to higher selectivity towards the cytoplasmic ribosome. 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.[14]

1.5 Pharmacology Studies

A series of preclinical studies has been conducted to evaluate the primary pharmacodynamics of ELX-02 in several genetic disease indications including CF, cystinosis, Duchenne muscular dystrophy, Mucopolysaccharidosis type 1, and Rett syndrome. This includes *in vitro* [organoid, human bronchial epithelial (HBE) cells, Fischer rat thyroid (FRT) cells, plasmid-based] and *in vivo* models of CF. Likewise, for cystinosis, both *in vitro* (fibroblast, plasmid-base) and *in vivo* models have been evaluated. These studies demonstrate that ELX-02 induces functional restoration of CFTR across the most common nonsense mutations in CF and cystinosis and estimate a weekly therapeutic exposure range of 20 to 190 µg*hr/mL.

In Vitro Efficacy Studies

ELX-02 has been evaluated across multiple *in vitro* model systems and demonstrates consistent, dose-dependent evidence of activity against several nonsense mutations.

ELX-02 read-through of the Col4A5 R1563X nonsense mutation that causes Alport syndrome was demonstrated in a reporter assay expressing Col4A5 fused to a C-terminal NanoLuc reporter. ELX-02 treatment significantly increased the read-through of Col4A5 R1563X reporter by 7-to10- fold.[26]

In cystinosis patient fibroblasts homozygous for the *W138X* mutation, incubation of ELX-02 at escalating dose for 72 hours resulted in reduced half-cystine levels (at ≥ 100 µg/mL) up to normal levels. ELX-02 also significantly increased *CTNS* mRNA levels (≥ 200 µg/mL) by 2.5- to 3.5-fold.

ELX-02 has also been evaluated across multiple cystic fibrosis (CF) *in vitro* model systems and demonstrates consistent, dose-dependent evidence of activity against the most common *CFTR* nonsense mutations. These CF models include reporter and overexpression systems, patient-derived primary HBE cells and patient-derived organoids. The results of these models have been favorably correlated with *in vivo* human clinical results.

> Reporter and Overexpression Systems: Initially, ELX-02 mediated read-through of CF nonsense mutations was demonstrated in a luciferase reporter system supporting that CF nonsense alleles were amendable to read-through. Next, ELX-02 demonstrated an increase in functional CFTR by increasing stimulated CFTR current using the FRT cell model overexpressing either *G542X* or *R1162X* bearing *CFTR* transgenes. These models supported our evaluation of patient-derived cells.
>
> Patient-derived Organoids: An organoid is a three-dimensional, multi-cellular *in vitro* system derived from stem cells isolated from rectal biopsies. Similar to measuring CFTR conductivity in other systems, such as HBE cells, CFTR activity is stimulated in the organoid system with forskolin to produce cyclic adenosine monophosphate, a signaling molecule that stimulates

CFTR channel activity. Organoids derived from nonsense mutation CF patients lack apparent swelling in this system due to their lack of functional CFTR. These studies demonstrate that across the most common CF causing nonsense alleles (*G542X*, *W1282X*, *R553X*, *R1162X*, and *E60X*) accounting for >75% of the CF nonsense allele population, ELX-02 significantly increases CFTR function in a dose dependent fashion. While approved CF therapies lack efficacy against nonsense CF mutation, organoids with responsive mutations to approved therapies (e.g. *G551D, F508del*) demonstrate activity in the organoid swelling assay in a manner that correlates with clinical responsiveness in informative endpoints such as FEV1 and sweat chloride change. When considering the ELX-02 results with the correlation reports of other approved molecules in this assay, the response mediated by ELX-02 read-through meets or exceeds the activity necessary to significantly improve FEV1 and sweat chloride concentration. Additionally, ivacaftor in combination with ELX-02 further increased CFTR channel activity supporting that the read-through CFTR product may be potentiated.

In Vivo Efficacy Studies and Therapeutic Exposure Estimates

ELX-02 pharmacokinetic properties drive its pharmacologic activity. Since it is an aminoglycoside, excellent systemic exposure ca be achieved along with elevated exposures in kidney. This has driven the exposure estimates in different diseases.

A cystinosis nonsense mouse model, $Ctns^{Y226X/Y226X}$, was evaluated for reduction in cystine accumulation.[15] *In vitro* evaluation of $Ctns^{Y226X/Y226X}$ mouse embryonic fibroblasts demonstrated significant reduction in cellular cystine accumulation within 24-hours using 100-400 µg/mL ELX-02. Mice with the same genetic background were treated with ELX-02 at 10 mg/kg or 30 mg/kg subcutaneously bi-weekly for 3 weeks (7 total injections). In mice, as expected, there were higher levels of ELX-02 in kidney versus plasma. After treatment, ELX-02 reduced kidney cystine levels by 30% in treated animals compared to untreated animals.

Figure 2: Kidney Concentration of Drugs vs Time Following Single and Repeated Subcutaneous Administration of ELX-02 (10 mg/kg) to CtnsY226X/Y226Y Mice

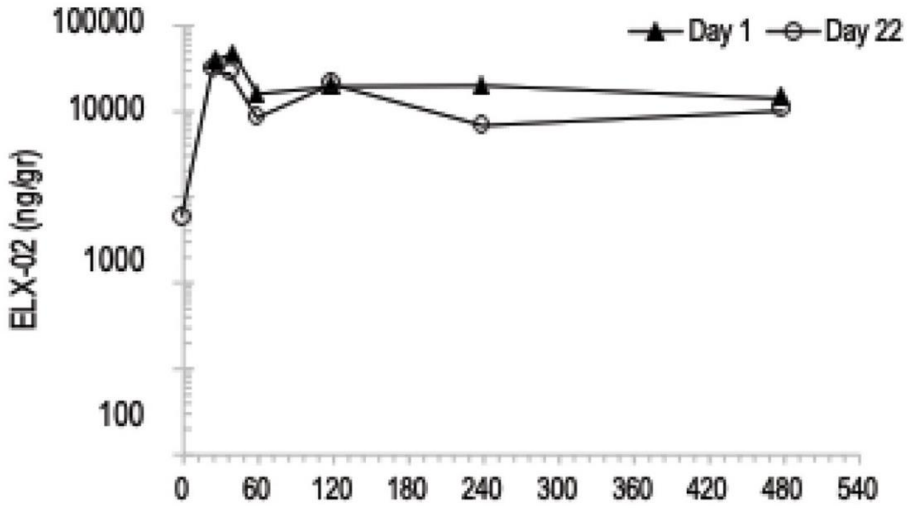
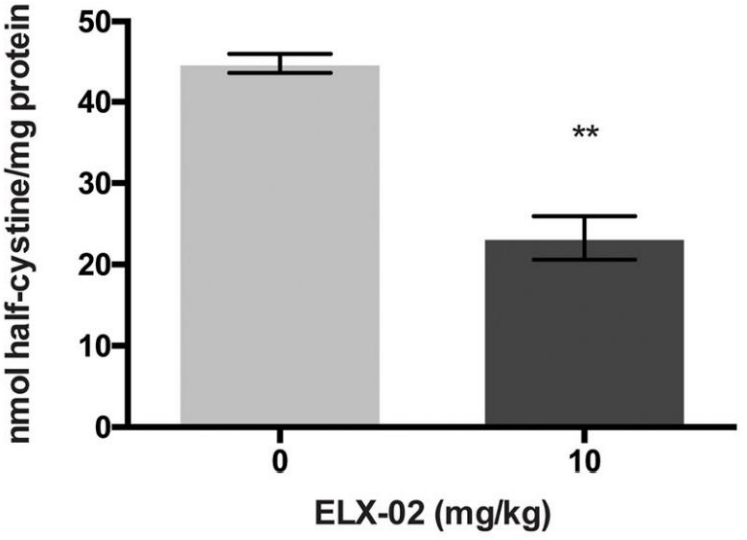


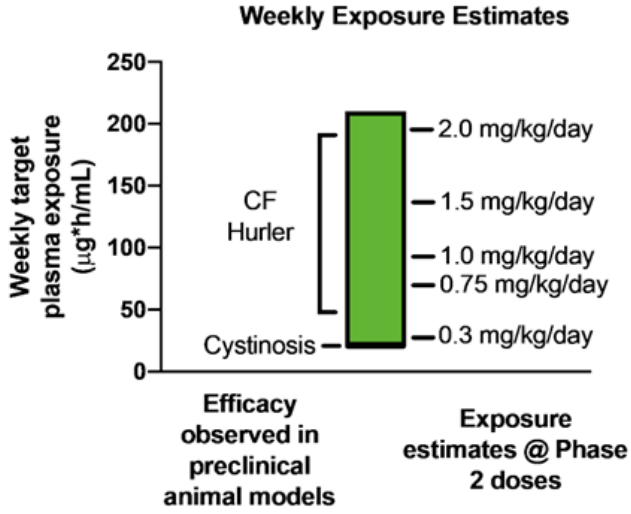
Figure 3: Effect of ELX-02 in CtnsY226X/Y226Y Mice



ELX-02 read-through was also evaluated using a *CFTR* knockout mouse model that expressed a human *G542X CFTR* transgene and the results of these studies show that ELX-02 restores CFTR function.[27, Eloxx Report CF-01] In these studies, the effect of ELX-02 on CFTR activity was tested in *Cftr* knockout mice expressing a human *CFTR-G542X* transgene under intestine-specific rat fatty acid binding protein (referred to hereafter as *Cftr*$^{-/-}$ *hCFTR-G542X* or *G542X* transgenic mice).[28,29] Mice were administered ELX-02 (30 and 60 mg/kg) by SC injection once daily for 14 days. CFTR activity was then assayed by Ussing chamber analysis to determine whether cAMP-stimulated transepithelial short circuit currents (I_{sc}) could be detected. Significant increases in forskolin-stimulated I_{sc} were observed in *Cftr*$^{-/-}$ *hCFTR-G542X* mice treated with 30 mg/kg ELX-02 (14.7 µA/cm^2). The increases in I_{sc} in these ELX-02-treated mice was 5.8% of the forskolin-stimulated I_{sc} observed in wild-type control mice, and the response to 30 mg/kg ELX-02 was roughly 2.5-fold greater than the currents observed in controls treated with the same dose of gentamicin.

Efficacious therapeutic exposure estimates are derived from a compilation of the *in vivo* nonsense models evaluated with ELX-02. This includes *Cftr*$^{-/-}$ *hCFTR-G542X* transgenic mice and a cystinosis mouse model.[27,15, ELX-01, Eloxx Report CF-01]. Inclusion of additional models serves to better estimate the full range at which tissue level read-through has been observed with ELX-02. Kidney level efficacy in a cystinosis mouse model was observed at a weekly plasma exposure of 20 µg*hr/mL while efficacy was observed between 47.5 and 190 µg*hr/mL in the gut of the *Cftr*$^{-/-}$ *hCFTR-G542X* transgenic model. The targeted efficacious dose for Alport is similar to cystinosis as both are diseases of the proximal nephron in the kidney.

Figure 4: Relationship Between Weekly Target Plasma Exposure in Preclinical Animal Models and Estimated Exposures of ELX-02 in Phase 2 Clinical Studies



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

1.6 ELX-02 Clinical Studies

1.6.1 Clinical Studies in Healthy Volunteers

Eloxx Pharmaceuticals has completed three studies in healthy volunteers:

- EL-001 and EL-006 were two monocentric, randomized, double-blinded placebo-controlled single ascending dose (SAD) studies.[30]
- EL-002 was a Phase 1, randomized, double-blinded, placebo-controlled, third party open, multiple ascending dose (MAD) escalation study.

Both SAD studies evaluated single doses of ELX-02 between 0.3 mg/kg and 7.5 mg/kg (N=40) or placebo (N=20) in a total of 60 normal volunteers and characterized general and specialized safety parameters and PK. ELX-02 was generally well tolerated, showed typical pharmacokinetic parameters for an aminoglycoside, and showed an acceptable safety profile without severe or serious study drug-related adverse events (AE).

The MAD study EL-002 evaluated the safety, tolerability and PK of subcutaneously administered ELX-02 in independent consecutive cohorts of healthy participants. The study was designed to include 7 cohorts of 9 participants (2:1 active study drug to placebo). Doses ranged from 0.1 mg/kg to 5.0 mg/kg SC twice a week over 29 days for a total of nine doses. The 1.0 mg/kg and 2.5 mg/kg doses were administered at final injection concentrations of 100 mg/mL and 50 mg/mL while other doses levels were administered at 50 mg/mL. A total of 41 participants received ELX-02 and 21 participants received placebo in the study. ELX-02 demonstrated dose-proportional PK in this study, with no evidence of accumulation for up to 29 days of bi-weekly administration. ELX-02 was generally well tolerated, there were no reported SAEs or renal findings.

Eloxx Pharmaceuticals has completed two other clinical trials:

- EL-008 – A Phase 1 study in participants with various severities of renal dysfunction and healthy volunteers. In this study, the mean plasma concentration profiles for Group 1 (mild

renal impairment, eGFR between 60 and 89 mL/min/1.73m^2) and Group 4 (control, normal renal function, eGFR ≥90 mL/min/1.73m^2) were nearly identical. Mean maximum plasma ELX-02 concentrations were higher and mean terminal elimination half-life was longer in Group 2 (moderate renal impairment, eGFR between 30 and 59 mL/min/1.73m^2) and Group 3 (severe renal impairment, eGFR <30 mL/min/1.73m^2, not undergoing dialysis) versus Group 4. Mean $t_{½}$ values were similar at 2.8 and 3.3 h for normal renal function and mild renal impairment; half-life increased to 6.4 and 21.2 h in moderate and severe renal impairment, respectively. Renal clearance of ELX-02 showed similar trends as plasma, with decreasing clearance as the severity of renal impairment increased.

- EL-003 – A Phase 2 study in patients with nephropathic Cystinosis. This study was discontinued due to non-safety reasons and did not proceed with the second cohort as planned in the original protocol. Once daily SC administration of ELX-02 targeting a plasma exposure of 95 µg*hr/mL/week for seven days (Treatment Period 2) in the first cohort resulted in a measurable percent reduction in white blood cell (WBC) cystine levels following seven days of treatment with ELX-02. Following 14 days of treatment at 190 µg*hr/mL/week (Treatment Period 3), two out of three participants showed a reduction in WBC cystine levels. During the hiatuses between Treatment Periods 1 and 2, and 2 and 3, the Day 1 pre-dose WBC cystine levels continued to increase above the pre-treatment baseline, which made interpretation of the results challenging. Therefore, this study was discontinued. There were no treatment-related SAEs. All TEAEs were mild or moderated in severity.

There are two ongoing Phase 2 clinical trials in patients with Cystic Fibrosis (Studies EL-004 in Israel, Germany and Australia and EL-012 in the United States and Canada). Overall, the ongoing Phase 2 studies for ELX-02 are considered to provide an adequate safety profile for CF patients. The adverse events experienced reflect the underlying disease state.

In conclusion, the overall safety profile of ELX-02 is adequate and manageable. The most common TEAE is injection site reaction which can be delayed by several days and the most severe reactions observed to date may require weeks to months to completely resolve. There have been no clinically meaningful changes in kidney function or hearing.

1.6.2 Clinical Safety in Healthy Volunteer Studies

Studies EL-001 and EL-006 (Single Ascending Dose or SAD)

Over the dose ranges tested (0.3 mg/kg to 7.5 mg/kg), ELX-02 was shown to be safe and generally well tolerated. For the pooled data of both studies, the most common treatment-emergent adverse events (TEAEs) occurring in ≥ 3 participants were injection site reactions (6 participants [10%]), headache (5 participants [8%]), injection site erythema (3 participants [5%]), and ear discomfort (3 participants [5%]). All of these TEAEs were considered mild in severity except for one case of moderate headache (placebo) and one event of high frequency threshold shift (5 mg/kg dose) on pure tone audiometry (PTA). This moderate AE of abnormal acoustic stimulation test was recorded in one participant on Day 8 after administration of ELX-02 5.0 mg/kg SC. The AE was considered possibly related to study drug by the Investigator, who also deemed it a serious adverse event (SAE). The AE was not resolved at the end of the study. This AE was evaluated in detail with the assistance of an independent audiology expert, and it was observed that there were random, bidirectional fluctuations in audiometry not consistent with aminoglycoside-induced ototoxicity, and that they were likely the result of equipment mis-calibration and maintenance, improper audiophone placement and/or uncontrolled noise in the environment. Overall, the conclusion by the Sponsor was that the AE was unrelated to the study drug, and it did not meet the criterion for SAE.

Study 002 (Multiple Ascending Dose or MAD)

ELX-02 was generally well tolerated, there were no reported SAEs or renal findings. Thirty-four (82.9%) participants in the ELX-02 treatment groups and 9 (42.9%) in the placebo groups experienced TEAEs related to the study drug, the majority of which were injection site reactions. All TEAEs were mild or moderate in severity, and none were classified as serious. The duration of observed injection site reactions was reduced from 28 days to 12 days for the 1 mg/kg dose and from 45 days to 19 days for the 2.5 mg/kg dose when the final injection concentration was reduced from 100 mg/mL to 50 mg/mL. One of 12 participants who received 2.5 mg/kg of ELX-02 and 3 of 6 participants who received 5.0 mg/kg of ELX-02 had significant hearing threshold shifts. The threshold shifts were largely confined to the high frequency range (10-16 kHz), above the conventional range (0.25-8 kHz) needed for normal human communication. No irreversible permanent and stable or progressive hearing threshold shift, or clinical impact was observed in any participant, nor did any participant demonstrate significant tinnitus or vestibular disorders that are commonly affiliated with ototoxicity. While the threshold changes were inconsistent with aminoglycoside induced ototoxicity in the participant who received 2.5 mg/kg, ototoxicity at the 5.0 mg/kg twice weekly dose could not be completely ruled out because the partially to fully reversable hearing threshold shifts in the very high frequency range in two participants were consistent with early ototoxicity. In Cohort 7 (for the 5.0 mg/kg group), 2 of 3 participants received placebo also experienced threshold changes, indicating that some variability in the audiometry test results may have contributed to the findings.

Study 008 (Renal Impairment Study)

ELX-02 was generally well tolerated following a single SC dose of 1.0 mg/kg. Overall, 6 participants had a total of 11 TEAEs. Five (20.8%) participants had TEAEs that were considered related to study drug. All TEAEs were classified as mild in severity. There were no deaths or SAEs reported in the study. None of the TEAEs lead to study discontinuation.

A majority of participants (83.3%) in the placebo group and 1/6 participants (16.7%) in Group 3 experienced at least 1 TEAE. All TEAEs were classified by the investigator as being related to the study drug. The most frequently reported TEAE was injection site erythema [observed in 4/6 (66.7%) participants in the placebo group and 1/6 (16.7%) participants in Group 3.

Study 003 (Study in Patients with Nephropathic Cystinosis)

In the cohort of three adult participants (≥18 years old with eGFR ≥40 mL/min/1.73m^2) in this study, ELX-02 was generally well tolerated following SC injection of ELX-02. Overall, a total of 16 Aes were reported in the three participants treated in the study. All AEs were considered mild (Grade 1) in severity, except for two events (choking, anemia) that were considered moderate (Grade 2) in severity. All events were resolved.

There were no adverse events of special interest (nephrotoxicity, ototoxicity, intolerable injection reactions) reported. There were 2 serious AEs (choking and allergic reaction to Venofer®) in one participant (101-001). The episode of choking related to underlying disease occurred during the screening period and the drug hypersensitivity reaction to Venofer® occurred during the hiatus between Treatment Periods 2 and 3. Both of these events resolved without complication and were not related to ELX-02. There were no TEAEs leading to death or study discontinuation.

1.6.3 Pharmacokinetics in Healthy Volunteer Studies

Studies EL-001 and EL-006 (Single Ascending Dose or SAD)

ELX-02 given SC was rapidly absorbed with a time to maximum concentration (t_{max}) of about 0.5 to 1.0 hr. $AUC_{0\text{-}inf}$ changed linearly with ELX-02 dose (24-fold increase for a 25-fold dose increase) and maximum plasma concentration (C_{max}) changed in a quasi-proportional way with ELX-02 dose as significant dose effects were seen (17-fold increase for a 25-fold increase). The apparent terminal half-life ($t_{1/2}$) was 2 to 8 hr 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 hrs.

Study EL-002 (Multiple Ascending Dose or MAD)

In Study EL-002, ELX-02 was rapidly absorbed following SC administration to healthy participants across a dose range of 0.1 to 5.0 mg/kg, with median t_{max} ranging from 45 minutes to 1-hr post-dose. Plasma ELX-02 exposure was dose proportional across the dose ranges evaluated, and no evidence of accumulation was observed following twice-weekly administration for up to 9 doses over 29 days. Volume of distribution was high, suggesting distribution beyond the blood compartment. Systemic ELX-02 was rapidly excreted in urine, with approximately 76 to 94% of the administered dose excreted within 12 hrs post-dose. Both plasma and renal clearance were high and independent of dose, consistent with the short elimination half-life of approximately 2 to 3 hrs. Plasma ELX-02 exposure was generally similar following SC administration of either a 50 or 100 mg/mL diluted injection solution, indicating no formulation-related differences in PK.

1.6.4 Pharmacokinetics, Exposure and Daily Dosing in Alport Patients

ELX-02 is an aminoglycoside which has a preferential uptake in the kidney. Megalin is a polybasic transporter which uptakes aminoglycosides (such as ELX-02). Due to the high expression of Megalin in the kidney (podocytes and proximal tubules) and the inner ear, a significantly lower dose of ELX-02 is required for treatment of Alport patients. The PBPK model developed from previous EX-02 PK studies predicts 52 times higher ELX-02 exposure in the kidney compared to the plasma at an ELX-02 SC dose of 1 mg/kg. A dose of 0.75 mg/kg is expected to be in the therapeutic range and will require only one SC injection daily to achieve consistent exposures.

1.6.5 Nonclinical Safety Studies

In a core battery of safety pharmacology studies, ELX-02 had no effect on cardiovascular, neurobehavior, or respiratory functions and no off-target pharmacological activity, or antibacterial activity. It displayed a short plasma half-life, long tissue half-life, with linear dose-dependent PK in mice, rats and dogs, dose proportional exposure in mice, rats, and dogs, and near 100% bioavailability between SC and IV dosing. ELX-02 does not undergo hepatic metabolism and is excreted unchanged in urine; tissue exposures were significantly longer than plasma exposure. At pharmacologic doses it showed no accumulation in plasma, and relatively low accumulation in the kidney and cochlear tissue. It also has negligible *in vitro* plasma protein binding in rat, dog, and human plasma, and high binding to brain tissue homogenates of CD-1 mice.

- In definitive repeat-dose toxicity studies using adult rats and dogs, ELX-02 administered in SC doses twice weekly (BIW) over a 28-day period had little or no effect on body weight, food consumption, clinical signs of toxicity, ophthalmology, cardiovascular parameters, or hematology or coagulation parameters. Clinical chemistry alterations reflected that of renal

dysfunction. In these 4-week toxicology studies, the exposures at the highest dose without renal findings were 87 µg*h/mL and 125 µg*h/mL in rats and dogs, respectively (corresponding to weekly AUC of approximately 174 and 250 µg*h/mL, respectively). The highest exposure at which no ototoxicity findings were observed was 413 µg*h/mL (corresponding to weekly AUC of approximately 826 µg*h/mL, respectively). In a confirmatory rat 4-week daily dosing tox study, the NOAEL was 30 mg/kg/day with a weekly exposure of 385 µg*h/mL, suggesting enhanced safety for daily compared to BIW dosing. Dosing. This result is consistent with a 29-day QD toxicity study in male beagle dogs daily ELX-02 at 13.5 kg/mg/day demonstrated no indications of nephrotoxicity with daily exposure of 38.4 µg*h/mL (weekly exposure of 268.8 µg*h/mL). Longer term GLP tox studies (rat 6-month tox and dog 9-month tox) identified similar NOAEL exposures with BIW dosing.

- The justification in patients <18 is based on the 13 weeks study in juvenile rats. In this GLP study, juvenile rats, PND 28, were given ELX-02 for 13 weeks and a subset of animals underwent a 28-day recovery period. Doses were given SC on a twice-weekly basis alternating between two injection sites located on each side of the scapular region and two sites on each side of the gluteal region of the animal. Dose levels were set at 0, 15, 30, and 45 mg/kg/dose (MPI Report 2490-002) based upon three assumptions: no adverse renal findings in adult rats at 60 mg/kg/dose for 4-weeks (Aptuit Report VPT4700); increased exposure duration may result in target organ toxicity at lower dose levels; and anticipated greater sensitivity to aminoglycosides in juvenile animals.

  There were no ELX-02 related adverse findings on survival, functional observational battery evaluations, body weight, body weight change, food consumption, ophthalmoscopic evaluations, motor activity, learning and memory, bone length or density, clinical pathology (hematology, clinical chemistry, coagulation, or urinalysis), organ weights, or neurohistopathological evaluations at any ELX-02 dose level. Furthermore, there were no adverse ELX-02 related effects on sexual maturation, reproductive and fertility indices, maternal uterine examinations, or sperm evaluations at any dose level. ELX-02-related non-adverse observations of scabbed area and red discoloration of the skin were evident in both genders at all dose levels. These changes correlated with macroscopic observations of skin abrasions/scabs at or around the lumbar injection sites in males at ≥30 mg/kg/dose and in females at 45 mg/kg/dose. These changes also correlated with microscopic findings of myofiber degeneration in the cutaneous trunci muscle, hemorrhage, SC edema, SC fibrosis, and mononuclear cell infiltration in the subcutis or muscle. There were occasional findings at the injection sites that were present in control animals and treated animals, indicative of a dosing procedure-related effect which showed a trend to recovery throughout the recovery period. At the terminal necropsy, minimal tubular degeneration and apoptosis in one kidney was observed in one female given 45 mg/kg/dose. These findings were multifocal and were characterized by cytoplasmic vacuolation and individual tubuloepithelial cell necrosis in several proximal tubules within the outer medulla. There were no findings in recovery animals. The findings were not considered adverse based on the minimal severity and the abundance of unaffected parenchyma.

As there were no adverse ELX-02-related effects in this study, the NOAEL was the highest dose tested, 45 mg/kg/dose, with a corresponding C_{max} of 68 and 67 µg/mL (male/female, respectively), and an $AUC_{0\text{-}24\ hr}$ of 63 and 58 µg*hr/mL (male/female, respectively, on Day 91.

1.7 Study Design

This is a Phase 2 open label pilot study to evaluate the safety and efficacy of subcutaneously administered ELX-02 in patients with Alport Syndrome with Col4A5 and Col4A3/4 nonsense mutation.

1.7.1 Objectives

Primary objective: Safety and tolerability of ELX-02 will be evaluated in this trial.

Secondary objectives: The readthrough effect of ELX-02 in expression of Col IV will be evaluated in kidney and ear. The expression of the Col IV in the kidney will be evaluated functionally by the changes in proteinuria and hematuria, two main early hallmarks of kidney disease in AS. Histologically, the expression of Col IV will be evaluated by expression of Col IV in the basement membrane. While the focus of the trial is to evaluate the efficacy in the kidney, and as such the patients are not selected based on their auditory phenotype. However, it is expected that most of the participants to have auditory phenotypes. The evaluation of the hearing of the participants is an exploratory endpoint.

1.7.2 Rationale for Patient Selection

Alport patients with nonsense mutations in Col4A5, Col4A4, and Col4A3 can benefit from treatment with a nonsense readthrough agent that can functionally restore Col IV as demonstrated in pre-clinical experiments. Patients 6-30 years of age will be included in this trial.

Patients who harbor nonsense mutations have an early onset of the disease leading to ESRD at age 17.9-21.4 years (Col4A5 and Col4A4/Col4A3 respectively). Due to the early onset and rapid progression to ESRD, it is imperative to initiate treatment early to restore collagen IV function in these patients if there is a chance to delay disease progression. It is important to note that patients as young as 13.3 years old with Col4A3/A4 reach ESRD.[31,32]

Phase 1 and Phase 2 studies of ELX-02 in at least 126 adults who received at least one dose of ELX-02 has shown to be well tolerated with no serious ELX-02 related adverse events seen. In addition, rat juvenile toxicology study showed no adverse ELX-02-related effects up to 45 mg/kg twice weekly dose with a corresponding C_{max} of 68 and 67 µg/mL (male/female, respectively), and an $AUC_{0\text{-}24}$ hr of 63 and 58 µg·hr/mL (male/female, respectively, on Day 91.

1.7.3 Rationale for Major Assessments

Urine collection: Hematuria and proteinuria are the earliest manifestations of AS. Hence, the primary and secondary endpoints of this study are proteinuria and hematuria, respectively. It is hypothesized that expression of functional Col IV in patients with nonsense mutations due to readthrough induced by ELX-02 can partially restore glomerular integrity thus reducing dysfunction and further injury resulting in an improvement of proteinuria and hematuria. Effective Col IV expression may also be measured in urine cells to correlate with changes in hematuria and proteinuria. Urine collection is required to evaluate the efficacy of ELX-02 in this study.

Audiometric test: While the patients in this study are not selected based on their hearing status, due to the early onset of the of the manifestation of disease in AS patients with nonsense mutation, a large

number of the patients are expected to have sensorineural hearing problem. Restoring the expression of the Col IV may improve the hearing of the patients. This is a non-invasive assessment and is required for assessment of safety and efficacy in this study.

Kidney biopsy: In this pilot study, it is important to assess for a signal of clinical efficacy in a comprehensive manner. The first indication of drug effect in Alport patients due to readthrough across a nonsense mutation is the formation of full-length COL IV protein. To assess the molecular restoration of protein function, kidney biopsies will be performed and assayed for COL IV protein levels and structural changes upon COL IV restoration, if any.

1.7.4 Rationale for Dose and Dosing Schedule

ELX-02 will be administered at 0.75 mg/kg SC daily. Patients with cystic fibrosis have received the same or higher doses of ELX-02 in previous Phase 2 studies. Extensive pharmacokinetic experience across studies, physiologically based pharmacokinetic modeling and pre-clinical pharmacology has provided a firm basis for dose selection. It is important to note that physiologic based PK modeling shows a >50 times exposure in the kidney compared to plasma at 1 mg/kg dosing in human. This preferential kidney targeting of ELX-02 underlies the robust results seen in preclinical study in cystinosis with half-cysteine reductions in kidney and is also anticipated to drive favorable exposures in Alport kidneys (see Section 1.5).

The ELX-02 fixed dose level for this study is 0.75 mg/kg/day (5.25 mg/kg/week). This dose corresponds with the MAD study dose of 2.5 mg/kg BIW (5 mg/kg/week). Based on the MAD healthy volunteer data, the weekly exposure is expected to be about 65 µg*h/mL. This exposure reaches the target therapeutic range and its safety is supported by healthy volunteer data as well as animal toxicology data.

- In a Phase 1 study (EL-008) in participants with various severities of renal dysfunction and healthy volunteers, the mean ELX-02 plasma concentration profiles for those with mild renal impairment (eGFR between 60 and 89 mL/min/1.73 m^2) were nearly identical to those in the control group with normal renal function (eGFR ≥90 mL/min/1.73 m^2). Hence by inclusion of participants with eGFR ≥60 mL/min/1.73 m^2), no dose adjustment is needed.

Dosing Schedule

The apparent terminal half-life ($t_{1/2}$) of single ascending doses in healthy volunteers was 2 to 8 hr (0.3 mg/kg to 7.5 mg/kg), with similar pharmacokinetics and no accumulation in multiple ascending doses when administered twice weekly (BIW). Given the short $t_{1/2}$ in healthy volunteers, ELX-02 will be administered daily in this trial to ensure consistent daily exposure, and avoid any possible increased risk of nephrotoxicity associated with multiple daily doses (e.g. three times/day). Daily administration will also minimize the injection volume of each dose, thereby improving tolerability in comparison with the significantly larger injection volumes administered BIW in the Phase 1 studies.

Once daily dosing has been well tolerated at doses up to 3 mg/kg/day in CF patients. The participants in this study will receive a dose of 0.75 mg/kg/day. This provides adequate safety margin based on clinical and non-clinical data.

1.7.5 Risk Benefit Analysis

1.7.5.1 Risks Related to ELX-02

ELX-02-mediated Nephrotoxicity: Nephrotoxicity from aminoglycosides is a known toxicity, generally dose related, observed with repeated dosing (cumulative total dose) and generally reversible. However, the pattern of aminoglycoside nephrotoxicity is distinct from the manifestation of chronic kidney disease in Alport patients and can be evaluated based on the pattern of the decline of renal function. As described in Section 1.6.5, ELX-02 causes nephrotoxicity in preclinical animal studies at high doses and after repeated doses. The administration of 0.75 mg/kg SC is significantly below the upper limit of toxicity. In ongoing trials, ELX-02 is administered as high as 3 mg/kg in CF patients.

Based on the toxicology studies, the rat and dog NOAELs were associated with renal findings that were reversible with a lack of progression for renal toxicity following chronic administration. Consistent with Section 1.6.5., and as related to nephrotoxicity:

- the rat 6-month toxicology data identified NOAEL doses, with BIW dosing, corresponding to weekly exposures of 244 µg*h/mL (3.8x safety margin).
- the dog 9-month toxicology data identified NOAEL doses, with BIW dosing, corresponding to weekly exposures of 172µg*h/mL (2.7x safety margin).
- a confirmatory rat 4-week daily dosing toxicology study identified a NOAEL corresponding to 30 mg/kg/day and a weekly exposure of 385 µg*h/mL, suggesting enhanced safety for daily compared to BIW dosing, and which is about 6.1-fold greater than the exposure targeted in this study.
- a 29-day QD toxicity study in male beagle dogs dosed ELX-02 at 13.5 kg/mg/day and demonstrated no indications of nephrotoxicity with daily exposure of 38.4 µg*h/mL (weekly exposure of 268.8 µg*h/mL). These data correspond to a >4.1-fold safety margin compared to the exposure targeted in this study.

Overall based on rat and dog toxicology data the safety margin of 0.75 mg/kg SC QD dosing is at least 2.7x chronic dosing in beagle dogs in 9 months. In addition, the 26-week juvenile toxicology safety margin is approximately 2 (exposure of 122 µg*h/mL).

In addition to the toxicology data, as described in Section 1.6.3, single doses of 7.5 mg/kg (single dose C_{max} = 20 µg/mL and exposure of 95 µg*h/mL) were administered to healthy volunteers with no signals of nephrotoxicity (increases in serum creatinine or biomarkers of acute kidney injury [KIM-1 and clusterin]).

Participant exposure, safety and labs (every 2 weeks monitoring of renal function) will be carefully followed through all clinic visits during the Treatment Period as well as during the safety Follow-up Period after administration of the last dose (every month). While patients with Alport in this trial have chronic kidney disease, any acute worsening of the renal function will be further evaluated during this trial.

ELX-02-mediated Ototoxicity

Cochleotoxicity and vestibulotoxicity are class effects of aminoglycosides observed to a varying extent with different agents. However, the acuity of the effect is different from the clinical pattern of

Alport patients. In the preclinical studies with ELX-02, no ototoxicity was observed in rats exposed to ELX-02 up to 240 mg/kg BIW for 4 weeks. At this dose, the approximate weekly exposure was 826 µg*h/mL, which is approximately 11.8-fold higher than the expected exposure in this study.

The data observed in clinical studies to date is noted in Section 1.6.2. There have been no reports of vestibulotoxicity in any participants dosed with ELX-02 to date. For the purpose of cochleotoxicity monitoring, it should be noted that one participant in the SAD study receiving a single dose of ELX-02 at 0.3 mg/kg IV had a reversible high frequency threshold shift in one ear that was not considered related to ELX-02 by the Investigator, and another participant administered a single dose of 5 mg/kg had a high frequency threshold shift that showed bidirectional fluctuation and was attributed to methodological issues by an audiometry expert. These AEs were evaluated in detail with the assistance of an independent audiology expert, and it was observed that there were random, bidirectional fluctuations in audiometry not consistent with aminoglycoside-induced ototoxicity, and that they were likely the result of equipment mis-calibration and maintenance, improper audiophone placement and/or uncontrolled noise in the environment. Overall, these auditory-related events were considered not related to the study drug. In the EL-002 MAD study ELX-02 did not demonstrate evidence of ototoxicity in the 0.1, 0.3, and 1.0 mg/kg groups. One of the participants in the 2.5 mg/kg group had reversible, high-frequency threshold changes inconsistent with aminoglycoside induced ototoxicity. Furthermore, three participants had partially- to fully reversible hearing threshold shifts at the 5.0 mg/kg twice weekly dose level. At this top dose, the hearing threshold shifts in the very high frequency range (10-16 kHz), above the conventional range (0.25-8 kHz) needed for normal human communication, were clearly observed in two participants and partially in a third. No irreversible permanent and stable or progressive hearing threshold shift, or clinical impact was observed in any participant, nor did any participant demonstrate significant tinnitus or vestibular disorders that are commonly affiliated with ototoxicity. Two out of three placebo-treated participants in Cohort 7 (in the 5.0 mg/kg group) were also found to have significant hearing threshold changes, indicating some variability inherent in this audiometry assessment. Overall, while changes were inconsistent with aminoglycoside induced ototoxicity, ototoxicity at the 5.0 mg/kg twice weekly dose level could not be ruled out.

Participant hearing and audiometry will be carefully monitored at baseline, Days 30 and 60 of the Treatment Period, and Days 90, 120 and 150 of the safety Follow up Period. If any abnormalities are observed, they will receive close audiometry follow up. The administration of 0.75 mg/kg SC is substantially below the upper limit of toxicity. In addition, improvement or stabilization of hearing is expected in AS patients due to the expression of Col IV.

Potential for Neuromuscular Blockade

Use of ELX-02 should be avoided in participants with known neuromuscular disorders or those that are being given neuromuscular blocking agents because structurally similar aminoglycoside compounds have been associated with neuromuscular blockade. No preclinical or clinical data exist at present with ELX-02 to support or refute this effect.

1.7.5.2 Risks Related to Study Procedures

ELX-02-mediated Potential for Local Injection Site Reaction

Limited and tolerable injection site reactions have been observed with ELX-02 in preclinical and clinical studies to date, and consistent tolerability data are emerging from ongoing Phase 2 studies. The injection site reaction is dose dependent and is also impacted by the number of injections. In this

trial, the ELX-02 administration will be limited to one daily injection so the potential for injection site reaction is reduced. In addition, once daily injection is expected to result in improved tolerability.

Potential Risks Associated with Kidney Biopsy

Recent studies show that the rate of minor complications due to the biopsy procedure is 4.5-15.1% (hematuria, resolving hematoma, bleeding), and the major complication rate is consistently about 1.5% (active bleeding, arteriovenous fistula, complicated hematoma). The patients with major complication had abnormal baseline coagulation (abnormal PTT and PT) and severe anemia (Hb<10) and these patients will not be included in this study as they will not satisfy the inclusion/exclusion criteria. Furthermore, in this high-risk patients who are not candidates for the trial, the risk is operator dependent. In this study, kidney biopsies will be performed in major academic centers with operators having years of experience in performing biopsies thus reducing risks further.

1.7.5.3 COVID-19

ELX-02 is not expected to have immunomodulatory effects that would present an increased risk to participants enrolled in the study, either contracting or experiencing a more serious disease if infected with COVID-19. Eloxx Pharmaceuticals will assist, as needed, to clinical sites to apply site-level procedures regarding COVID-related precautions for patients and staff. The study visit schedule has also been carefully reviewed to minimize unnecessary visits and patient exposure.

Eloxx Pharmaceuticals will continue to assess risk during the course of the clinical study due to COVID-19 impact. This includes risk assessment for the overall study, as well as at the country/regional levels since changes/adaptations may be needed at a local level depending on the local COVID-19 situation. This may include impacts on visit schedules, assessments performed in-person or virtually, and alternative options for IMP re-supply for patients.

Further COVID-19 information can be found in Section 10.6.

1.7.5.4 Benefits

ELX-02 is being developed for patients with genetic disorders caused by nonsense mutations, including patients with Alport Syndrome. Multiple *in vitro* and *in vivo* models support the mechanism of action and read-through potential of ELX-02, supporting the potential efficacy of ELX-02 in patients with Alport Syndrome caused by nonsense mutations. Despite the significant morbidity and mortality of AS, there is currently no approved drug for Alport patients.

ELX-02 has a potential to induce the production of functional Col IV in patients with AS harboring nonsense mutations. This induction of functional Col IV is based on its unique mechanism of action and unique pharmacology of preferential absorption in the proximal nephron of the kidney (including the glomerulus) and the inner ears. Similar strong absorption of ELX-02 is known to occur in the inner ear. This potential functional replacement of Col IV early in the course of the disease prior to the deleterious effects of kidney fibrosis may slow down or halt the progression of the disease. For example, treatment with a readthrough agent, gentamicin, an agent with 10-fold lower readthrough than ELX02, in patients with recessive dystrophic epidermolysis bullosa harboring COL7A1 nonsense mutations led to replacement of Collagen VII at the dermo-epidermal junction and induced wound healing.[27]

1.7.5.5 Overall Risk Benefit Analysis

At this time, there is no treatment for AS patients. In general, the most common expected risk associated with the administration of ELX-02 is injection site reaction based on treatment of adults.

However, this is the first-time children as young as 6 years old will be exposed to ELX-02. It is important to note that ELX-02 is a non-antibiotic aminoglycoside, and no additional risk is known in pediatric population for this class of molecules. Consistent with this, adult safety data from prior ELX-02 studies and non-clinical juvenile toxicology study, no specific pediatric monitoring is required. Renal and cochlear/vestibular monitoring, as has been specified for adults, will also apply to children. The other risk associated with this trial is the performance of kidney biopsy which is significantly de-risked by the patient population and the procedure being performed in expert major academic centers. In terms of benefit, AS patients may have renal and sensorineural hearing stabilization after treatment with ELX-02. Based on the overall safety profile of ELX-02 and the current limited pilot study in AS patients, the overall risk benefit analysis justifies conducting this trial.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objectives

1. To assess the safety and tolerability of ELX-02 administered subcutaneously (SC) in patients with Alport Syndrome with Col4A5 and Col4A3/4 nonsense mutation.

2.1.2 Secondary Objectives

1. To assess the effect on ELX-02 in proteinuria.
2. To assess the effect on ELX-02 in expression of Col IV protein in kidney.
3. To assess the effect on ELX-02 in hematuria.

2.1.3 Exploratory Objectives

1. To assess the effect of ELX-02 on potential improvement in hearing of the patients with compromised hearing.
2. To assess Col IV protein levels in urine and or kidney cells, if warranted.

2.2 Endpoints

2.2.1 Primary Endpoints

1. The incidence and characteristics of adverse events (AEs). Other safety assessments will include injection site reactions (DAIDS Grading Scale) and changes from baseline in vital signs, physical examination, electrocardiograms, blood and urine safety laboratory tests, audiometric testing (including high frequency audiometry) and Tinnitus and Dizziness status.

2.2.2 Secondary Endpoints

1. Change from baseline to EOT and EOS in proteinuria.
2. Change from baseline to EOT in Col IV protein expression in renal biopsy
3. Change from baseline to EOT and EOS in hematuria.

2.2.3 Exploratory Endpoints

1. Change in audiometric testing (including high frequency audiometry) in individuals with baseline abnormalities.
2. Change in Col IV protein levels in urine and/or kidney cells based on changes in urine.

3 STUDY DESIGN

This is a Phase 2 open label pilot study to evaluate the safety and efficacy of subcutaneously administered ELX-02 in patients with X-linked or autosomal recessive Alport Syndrome with Col4A5 and Col4A3/4 nonsense mutation.

In total, up to 8 participants, with a minimum of 3 adults, will be enrolled in the trial. In case of a screen failure, participants may be re-screened at the discretion of the Investigator.

The study will be comprised of the following periods for each participant:

- a Screening period of up to 6 weeks (42 days)
- a total Treatment Period of 8 weeks (60 days)
- a safety/efficacy Follow-up Period of 12 weeks (90 days) after the last treatment.

The Treatment Period will be a treatment of ELX-02 0.75 mg/kg SC QD for 8 weeks (60 days).

During the treatment period, participants will receive their first dose of study drug at the clinic and have study assessments done (Day 1). Participants will return to the clinic for a study visit every 2 weeks for dosing, safety monitoring, efficacy and return/re-dispensing of study drug ELX-02. Outside of study visit days, treatment will be self-administered (or by a trained care provider) at participant's home. Participants (and/or caregivers) will be instructed on self-administration to ensure safety and compliance.

The study will initially enroll participants 12-30 years old only. Once the first two participants of ages 12-18 years complete 4 weeks of study drug treatment, a DSMB will evaluate the safety and PK data of these two sentinel participants. Following DSMB review and decision on the safety profile of the study drug, participants younger than 12 years of age (6-11 years old) can be dosed with the study drug.

If needed, the DSMB will review reported potentially related serious adverse events (SAEs) leading to discontinuation or study stoppage as they are reported. The determination of relatedness will be made by the treating investigator (Section 8.9).

At home, ELX-02 daily dose should preferably be taken at approximately the same time every day. Subcutaneous injection of ELX-02 is to be administered to the abdominal area or any area with a significant fat deposit (such as the upper thigh). Caution should be taken to ensure the injection is not administered into muscle.

A participant should not have elective injectable aminoglycoside during days of active ELX-02 treatment and at least 14 days before or after ELX-02 treatment. For any unplanned/urgent injectable aminoglycoside use, ELX-02 administration should be suspended and restarted 14 days after aminoglycoside treatment completion.

3.1 Stopping Rules

All participants will proceed through the entire Treatment Period unless they meet an individual stopping criterion (see Section 3.1.1), or the study meets the stopping criteria (see Section 4.8).

3.1.1 Dose Stopping in an Individual Participant

- Injection site reactions:
    - The occurrence of a DAIDS grade 3 or higher injection site reaction

- Nephrotoxicity as defined by Grade 2 Common Terminology Criteria for Adverse Events (CTCAE Version 5) based on serum creatinine
- Participants who evidence a Grade 2 CTCAE hearing impairment in the conventional frequencies or high frequency threshold shift meet stopping criteria for suspected ototoxicity. For adults, this is specified as a worsening shift from baseline of >25 dB averaged at 2 contiguous test frequencies in at least one ear, or at least 5-point worsening on THI or DHI questionnaires and category change. For pediatric participants, this is specified as a worsening shift from baseline of >25 dB averaged at 2 contiguous test frequencies in at least one ear including a worsening shift of > 20 dB at 4 kHz in at least one ear.
- Any other adverse event (AE), which in the opinion of investigator puts the participant at undue risk

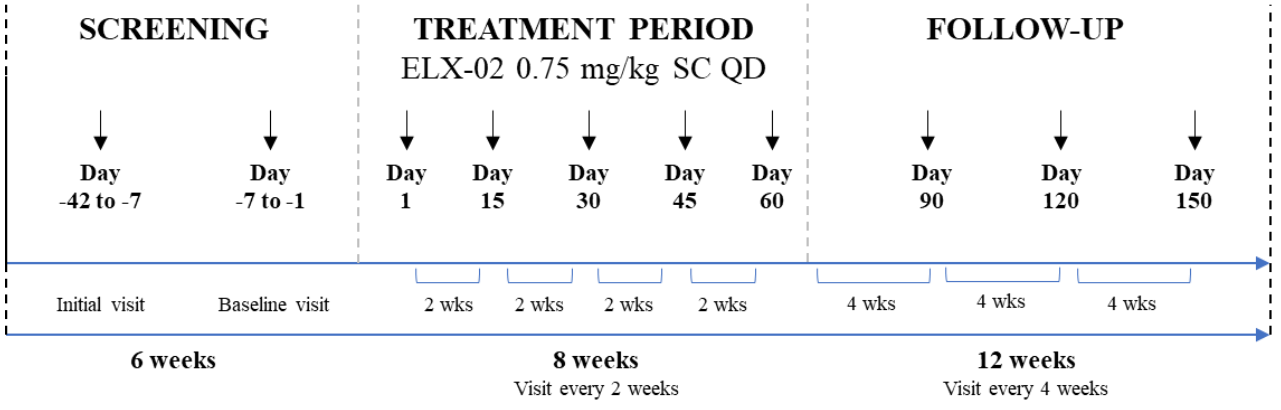
If it is determined following a thorough review that the initial assessment was incorrect, treatment of the participant may be resumed. This determination will be made by the DSMB in agreement with the Sponsor.

Participants who withdraw prior to study completion for non-safety reasons (e.g., personal reasons or non ELX-02 related AEs) may be replaced upon approval of the Sponsor.

3.2 Study Design Schema

The design of the study is presented in Figure 5.

Figure 5: Study EL-014 Study Schematic



4 PARTICIPANT SELECTION

In total, up to 8 participants, with a minimum of 3 adults, will be enrolled in the trial. In case of a screen failure, participants may be re-screened at the discretion of the Investigator.

This study can fulfill its objectives only if appropriate participants are enrolled. The following eligibility criteria are designed to select participants 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 participant is suitable for this protocol.

Participant eligibility should be reviewed and documented by an appropriate member of the Investigator's study team before the participant is included in the study.

4.1 Inclusion Criteria

Participants must meet all of the following criteria to participate in this study:

1. Evidence of signed and dated informed consent/assent document(s) indicating that the participant (and/or their parent/legal guardian) has been informed of all pertinent aspects of the trial
2. Understands, and is willing and able to comply with all scheduled visits, treatment plan, laboratory tests, and other study procedures
3. Male and female participants
4. Ages between 6 years and 30 years
5. A confirmed diagnosis of X-linked or autosomal recessive Alport Syndrome with a documented nonsense mutation of Col4A5 in a male or nonsense mutation of Col4A3 or Col4A4 (male or female).
6. The nonsense mutation should be UAG or UGA
7. eGFR≥60 ml/min/1.73 m^2 (based on CKD-EPI for ages ≥18 and Schwartz formula for participants <18)
8. Urinary protein based on two spot urine collections [urine protein/creatinine ratio (UPCR) ≥ 500 mg/g]
9. Stable regimen of ACEi/ARB for 4 weeks before screening (unless there is a contraindication)
10. Must be willing to abstain from strenuous exercise during the 48 hrs prior study visits
11. Females of childbearing potential and males capable of fathering a child must meet the contraception requirements outlined in Section 6.3
12. Non-lactating females
13. Females on hormone replacement therapy (estrogen or progesterone) or contraceptive therapy must be stabilized on a product and dose for at least 30 days prior to Screening
14. Have not received systemic medications with potential to impair renal function on a frequent basis (e.g., nonsteroidal anti-inflammatory drugs [NSAIDs]) or with ototoxic potential (e.g., quinine or salicylates), or any injectable aminoglycosides for a period of at least 14 days prior to dosing

15. Negative human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), and hepatitis C virus antigen (HCV Ag) serology tests at Screening
16. No history of alcohol or drug abuse within the 6 months prior to Screening
17. Body Mass Index (BMI) of 19.0 to 30.0 kg/m^2 (inclusive). Participants with a lower BMI may be entered into the study at the discretion of the investigator following consultation with the Sponsor

4.2 Exclusion Criteria

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

1. Participants 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 participants who are Eloxx Pharmaceuticals employees directly involved in the conduct of the study
2. Participation in clinical study including administration of any investigational drug or device in the last 30 days or 5 half-lives (whichever is longer) prior to investigational product dosing in the current study
3. Use of prohibited medications as defined in Section 6.1 within the specified windows
4. History of any comorbidity which in the opinion of the investigator might confound the study or pose an additional risk in administering the study drug to the participant
5. History of any organ transplantation
6. Mutation consistent with autosomal dominant Alport Syndrome
7. Liver disease characterized by cirrhosis or portal hypertension. Participants with alanine aminotransferase (ALT), aspartate aminotransferase (AST), and/or a total bilirubin 3.0 times the upper limit of normal (ULN) will be excluded
8. History of congestive heart failure diagnosed clinically or with documented left ventricular ejection fraction (LVEF) $\leq$ 40%
9. Evidence or history of clinically relevant psychiatric condition
10. A positive urine drug screen (amphetamines, benzodiazepines, cocaine and opiates) at Screening
11. Screening supine 12-lead electrocardiogram (ECG) demonstrating any clinically significant findings as judged by the Investigator
12. Participants with any abnormalities in clinical laboratory tests at Screening, considered by the study Investigator as clinically relevant
13. Major surgery within 180 days (6 months) of Screening
14. History of dialysis
15. Known allergy to any aminoglycoside
16. Participants with any acute medical situation unresolved within 14 days of first dose that is considered of significance by the Investigator

17. Participants with >10 dB change in threshold between audiometric Initial test to Baseline test for the frequencies from 0.5 -12.5 kHz
18. Dizziness Handicap Inventory (DHI) score at screening ≥16 for adults, and active dizziness reported for pediatric participants.

4.3 Rater Qualifications

Audiometric and vestibular tests will be performed by an audiologist or ear, nose, and throat (ENT) specialist. More details are provided in Section 7.1.13.

4.4 Participant Identification

Each participant who has signed the informed consent will be assigned a unique study number.

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

4.5 Re-Screening of Participants

If dosing is not expected to occur within 42 days of initial Screening procedure for any reason, the following re-Screening assessments will be performed to allow his/her participation: weight with BMI, vital signs, physical examination, ECG, blood safety lab tests, blood test for pregnancy, general urinalysis and urine for proteinuria. Audiometry, Tympanometry, SRT, Otoscopy and THI/DHI questionnaires must be repeated if ELX-02 dosing cannot occur within 7 days of the baseline assessment (if already completed).

Participants who fail to meet the enrollment 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 participants will be withdrawn from the study and will not count towards the total enrolled or total eligible participants but can be rescreened once at the discretion of the Investigator.

4.6 Treatment Interruption and Continuation

If deemed necessary by the investigator, or requested by the participant, and approved by the Sponsor, the participant may have a treatment interruption. Reasons for this may include a medical reason unrelated to an adverse event (e.g, a planned procedure), or important social or administrative events, or the occurrence of an adverse event, due to the participants underlying disease state. The reason for the treatment interruption is to be documented.

If treatment is interrupted for up to 7 days inclusive, then treatment will resume from the same timepoint treatment was stopped. Dosing count will continue the day after last dose was taken. If treatment is interrupted for more than 7 days, then treatment will be restarted. Dosing will restart from Day 1 and the participant will complete a new 60-day Treatment Period.

4.7 Removal, Replacement or Early Withdrawal

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

The individual'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. Participant withdraws consent.
3. Female participant is pregnant.
4. Substance abuse during the study
5. AE or SAE meeting criteria for study drug-related event and meeting stopping criteria (See Section 3.1).
6. Participant is unwilling or unable to continue the study or is lost-to-follow-up.
7. Investigator decides that withdrawal from the study is in the best interest of the participant.
8. Participant needs medication not allowed in the protocol.
9. Any clinically significant change in participant's medical condition.

Participants who withdraw prior to study completion for non-safety reasons (e.g., personal reasons or non-ELX-02 related AE) may be replaced upon approval of the Sponsor.

4.8 Early Study Termination

The study may be discontinued prematurely at a study site 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. Administrative reasons.
4. Exposure of the participant to unacceptable health risks.
5. A regulatory authority decision.
6. Change in opinion of the Independent Ethics Committee (IEC)/Institutional Review Board (IRB).
7. Following DSMB recommendation.
8. At the discretion of the Sponsor (e.g., decision of the Sponsor to discontinue development of ELX-02 at any time).

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

4.9 Lost to Follow-up

A participant will be considered lost to follow-up if the participant misses 2 consecutive study visits and is subsequently unable to be contacted by telephone (3 documented attempts by telephone within 2 weeks following the second missed visit).

For participants considered lost to follow-up, the CRF must be completed up to the last visit performed.

5 STUDY TREATMENTS

For the purposes of this study, and per International Council for Harmonization (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). In this study, ELX-02 is considered an investigational product.

5.1 Participant Compliance

ELX-02 will be administered by the appropriately designated study staff at the investigational site, or at home, self-administered by the participant or a trained caregiver.

5.2 Investigational Product Supplies

5.2.1 ELX-02

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

ELX-02 will be labeled according to local law and regulatory requirements.

5.2.1.1 Dosage Form(s) and Packaging

TEST PRODUCT: The active ingredient (ELX-02) is a synthetic aminoglycoside, manufactured in compliance with GMP standards. The drug product is manufactured Additional manufacturer information is provided in the IMPD. The study drug is manufactured as a lyophilized powder for injection, to be reconstituted with 0.9% sodium chloride (NaCl) to a concentration of 50 mg/mL prior to dose administration. A schedule for dose volumes will be provided in the pharmacy manual.

5.2.1.2 Dispensing and Administration

A 9-day supply of ELX-02 will be dispensed to each participant at study visits during the Treatment Period (except for Day 60) for in-clinic administration and for home self-administration. Instructions for study drug handling will be provided to the participant/caregiver at the Day 1 visit and at following visits as needed. Additional study drug will be shipped to the participant's home between scheduled study visits to ensure a continuous supply of ELX-02 (detailed explanation of study drug dispensation will be described in the Pharmacy Manual).

ELX-02 will be administered to each participant at study visits during the Treatment Period by a designated study nurse or Investigator. Participants/caregivers will have injection administration training at the Day 1 visit, and at each following study visit as needed, to support self-administration at home.

The study drug will be administered as SC injection to the abdominal region around the umbilicus or to any area with a significant subcutaneous adipose tissue (e.g., thigh).

Details of each ELX-02 administration will be recorded by site staff when administered in the clinic and by the participant/caregiver in the participant diary when administered outside the clinic.

Unused and used vials from which the study drug was administered to the participants will be retained for dose confirmation. For details on the study drug accountability, refer to the pharmacy manual.

5.3 Missed Doses

If a participant misses a dose and recall the missed dose within 8 hours of their usual dosing time, then the participant should inject their daily dose on that day as soon as the participant recalls. If the participant recalls of the missed dose more than 8 hours after their usual dosing time, the participant should skip that dose and resume their normal schedule for the following dose on the next day.

5.4 Investigational Product Storage

ELX-02 will be stored at or below 25°C, and once reconstituted, the product should be stored in a refrigerator (2°C to 8°C).

Regular temperature logging of the study drug storage conditions at the clinic pharmacy should be performed. In case a deviation in storage conditions should occur, the clinic or pharmacy 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. 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 to the Sponsor 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.

5.5 Investigational Product Accountability

The investigative site must maintain adequate records documenting the receipt, use, loss, or other disposition of the investigational product supplies, including dispensing to and return from participants.

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

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

After the last visit of the last participant in the study (LPLV), any unused study drug will be returned to the Sponsor or destroyed at the clinic pharmacy 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 needles, syringes containing hazardous liquids, should be disposed of immediately in a safe manner, and therefore, will not be retained for study drug accountability purposes.

5.6 Destruction of Investigational Product Supplies

The Sponsor or designee will provide guidance on the destruction of unused investigational product (e.g., 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 Medications and Prohibited Medications

Participants must be on a stable regimen of ACEi/ARB for 4 weeks before screening (unless there is a contraindication).

Acetaminophen/paracetamol may be used at doses of ≤2 g/day. Herbal supplements should be discontinued at least 30 days prior to administration of the study drug.

A participant should not have elective injectable aminoglycoside, during days of active ELX-02 treatment, and at least 14 days before or after ELX-02 treatment, if possible. For any unplanned/urgent injectable aminoglycoside use, ELX-02 should be suspended and restarted 14 days after aminoglycoside treatment completion.

Co-administration of inhaled aminoglycosides are allowed during the study.

Use of daily scheduled ibuprofen (>200 mg/day) is not allowed during the study.

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

- Antimicrobials (such as other aminoglycosides, cephalosporins, clindamycin, polymyxin B) which may increase the risk of nephrotoxicity and/or neurotoxicity 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
- Immunosuppressive 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 agent with ototoxic potential
- Salicylates: an agent with ototoxic potential

6.2 Limit of Noise Exposure

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

In addition, participants will be required to wear silicone earplugs when exposed to louder than normal noise. Participants should avoid loud concerts, visiting a shooting range, and so on.

6.3 Contraception

All sexually active male and female participants should use one effective barrier method (a condom or a diaphragm) to avoid transfer of and exposure to ELX-02 to their partners. All male participants

who are able to father a child and female participants who are of childbearing potential and are sexually active and at risk for pregnancy must agree to use two highly effective methods of contraception consistently and correctly with their male or female partner throughout the study. All contraceptive methods must be used from Screening to at least 30 days after the last dosing for both female and male participants. The Investigator or designee, in consultation with the participant, will confirm that the participant has selected appropriate methods of contraception from the permitted list of contraception methods (see Section 6.3.1 below). In addition, the Investigator or designee will instruct the participant to call the site immediately if the selected contraception method is discontinued or if pregnancy is known or suspected in the participant or the participant's partner. All this will be documented in the participant's source documents.

Female participants that have not achieved menarche (has not had her first menstrual period) are considered not to be of childbearing potential only as long as they have not had their first menstrual period. Female participant who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea ≥12 consecutive months; or women on hormone replacement therapy with documented serum follicle-stimulating hormone level ≥35 mIU/mL), is considered of childbearing potential.

6.3.1 Birth Control Methods Which May be Considered as Highly Effective

For the purpose of this document, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods.

Acceptable contraceptive methods for females include the following:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - oral
    - intravaginal
    - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
    - oral
    - injectable
    - implantable
- Intrauterine device
- Intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Vasectomized partner (conducted at least 90 days before Screening, and documented azoospermia)
- Sexual abstinence (In the context of this document sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments and is the preferred and usual lifestyle of the participant).

Acceptable contraceptive methods for males include the following:

- Condom and spermicide
    - In countries where spermicide is not available, condom without spermicide will be considered acceptable.

    - Local regulations may require use of an additional acceptable method of contraception.
- Vasectomy 6 months or more previously, with a negative post-vasectomy semen analysis for sperm.
- Sexual abstinence (In the context of this document sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments and is the preferred and usual lifestyle of the participant).

Additional notes:

- Male and female participants who are not sexually active at the time of screening must agree to follow the contraceptive requirements of this study if they become sexually active with a partner of the opposite sex.
- Male participant must not donate sperm after the first dose of study drug, throughout the study, and for 30 days following the last dose of study drug.
- Female participants and female partners of male participants should not plan to become pregnant during the study through 30 days following the last dose of study drug.
- Female participants should not nurse a child from the start of study drug dosing through 30 days following the last dose of study drug.

7 STUDY ASSESSMENTS AND PROCEDURES

This study is a Phase 2 open label pilot study to evaluate the safety and efficacy of subcutaneously administered ELX-02 in patients with X-linked or autosomal recessive Alport Syndrome with Col4A5 and Col4A3/4 nonsense mutation.

In total, up to 8 participants, with a minimum of 3 adults, will be enrolled in the trial. In case of a screen failure, participants may be re-screened at the discretion of the investigator.

The study will be comprised of the following periods for each participant:

- a Screening Period of up to 6 weeks (42 days)
- a total Treatment Period of 8 weeks (60 days)
- a safety/efficacy Follow-up Period of 12 weeks (90 days) after the last treatment.

The Treatment Period will be a treatment of ELX-02 0.75 mg/kg SC QD for 8 weeks (60 days).

7.1 Study Assessments

The timing and types of assessments are specified in Table 1 below.

Table 1: Schedule of Procedures and Assessments

Period Identifier	Screening Period		Treatment Period					Follow-up		
Day in Study[1,17]	Day -42 to -7 Initial	Day -7 to -1 Baseline[16]	Day 1	Day 15	Day 30	Day 45	Day 60 EOT	Day 90	Day 120	Day 150 EOS
Allowed windows (±days)	--		+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3
Informed consent[2]	X									
Demographics	X									
Medical history[3]	X									
Inclusion/exclusion criteria	X		X							
Height	X									
Weight, body mass index	X		X				X			
Vital signs[4]	X		X	X	X	X	X			
Physical examination[5]	X		X	X	X	X	X			
12-lead ECG[6]	X						X			
Development Stage[7]			X				X			
Kidney Biopsy[8]	X						X			
Confirmation of nonsense mutation AS	X									
Blood safety lab tests (central)[9]	X		X	X	X	X	X	X	X	X
HIV, HBsAg, HCV Ab	X									
Urinalysis and urine pellet[10]	X	X	X	X	X	X	X	X	X	X
Proteinuria (UPCR) collection[11]	X	X	X	X	X	X	X	X	X	X
Serum FSH, LH (females)[12]	X									
Blood test for pregnancy[12]	X									
Blood collection for PK[13]			X	X	X	X	X			
Urine test for pregnancy[12]			X	X	X	X	X			
Urine for drugs of abuse	X									
Audiometry assessment[14]	X	X			X		X	X	X	X
Tympanometry[14]	X	X								
Speech Reception Threshold[14]	X	X								
Otoscopy[14]	X	X			X		X	X	X	X
Dizziness and Tinnitus status[14]	X	X			X		X	X	X	X
Clinic dosing			X	X	X	X	X			
Dispense study drug			X	X	X	X				
Injection administration training			X							
Evaluation of injection site[15]			X	X	X	X	X			
Return unused study drug				X	X	X	X			
Adverse events monitoring	◄ All visits ►									
Concomitant medications	◄ All visits ►									

1. In case of emergency, all site visits except Initial, Baseline and Day 1 visits, can be performed at home. The study drug can be shipped to the participant. UPCR can be performed at a central lab. Audiology assessments to be performed once site visits are resumed.
2. No invasive study assessments will be performed prior to signing Informed Consent/Assent.
3. Including Alport Syndrome history and historical lab values of proteinuria, creatinine, hematuria up to 5 years back. Participants that performed kidney biopsy in the last 5 years, will be asked to provide the biopsy sample for further analysis to evaluate collagen IV expression.
4. Heart rate (HR), blood pressure (BP), respiratory rate, and oral/tympanic temperature. Vital signs will be measured in supine position after at least 5 min rest. Vital signs will be performed before blood sampling collection. On dosing days, vital signs will be measured up to 90 min before dosing and 1hr ± 15 min post-dosing.
5. Detailed physical examination will be performed on Screening. On other visits, a brief physical examination is to be performed prior to dosing, focusing on CV, pulmonary, and hearing.
6. An ECG will be done at Screening and at 1hr ± 15 min post-dosing on Day 60. The 12-lead ECG will be performed before blood sampling collection.
7. Participants < 18 years will be evaluated for developmental stages based on Tanner stages.
8. Kidney biopsy is mandatory for adult participants (age determined per country regulation) and optional for pediatric participants at the Screening Period (after all eligibility assessments have been completed and patient eligibility has been confirmed) and on Day 60 ± 3 days.
9. Blood samples will be collected before dosing (see Table 2). Blood sample processing is described in detail at the lab manual.
10. Urine sample will be collected before dosing for urinalysis (gross, dipstick and microscopic) as well as urine pellet. Urine collected from participants at the day of the Initial visit as well as on the Day 30 and Day 60 visits, will be further analyzed for urine pellet. At the Baseline Visit, urine will be collected for urine pellet only. Urine sample processing is described in detail at the lab manual.
11. Two urine collections are needed for UPCR assessment at each visit. One sample will be collected at home the day before the visit and the second sample on the day of the visit. Both samples will be the collection of second void at each day after discarding the first morning void. Samples should be collected in separate cups, kept separately, and refrigerated until they are provided at the site. If second void on the day of the visit is collected at the study center, then it can also be used for urinalysis. At the initial visit, sample will be collected at the day of the visit at the study site and a second sample will be collected on the following day.
12. In female participants between the ages 12 years to 30 years of age. For female participants <12-year-old, no urine pregnancy tests are required. Pregnancy test will be conducted if investigator judges it to be necessary.
13. Pharmacokinetic (PK) blood samples will be taken pre-dose (up to 90 min before dosing) and after 1 hr ± 15 min post dosing at each site visit during the Treatment Period. 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.
14. During the Screening period, two sets of audiology assessments will be performed to address Exclusion Criterion #17: Initial assessments and Baseline assessments. Bone-conduction, SRT and Tympanometry will be performed at the Initial and Baseline assessments and must be repeated at other study visits only if there is any suspicion of a threshold shift or hearing loss on audiometry (≥ 15 dB at any frequency), or if requested by the Investigator. In any case of audiometry significant change criteria from Baseline on other visit days, a re-test must be performed after 15 min, or up to 24 hours. Dizziness and Tinnitus status will be evaluated by THI and DHI self-assessment questionnaires for adults. Pediatric participants will be questioned by the site staff about their tinnitus/dizziness condition, following by Pediatric DHI if dizziness status is active.
15. The injection sites will be assessed pre-dose (at all visits except for Day 1) and at 1 hr ± 15 min post dose.
16. Baseline visit must be performed within 7 days prior to dosing and at least 2 weeks after the Audiology Initial assessments were performed at the Initial Visit.
17. All assessments may be repeated at the investigator's discretion and Sponsor approval.

7.1.1 Screening Phase (Initial and Baseline Visits)

The Screening Phase of the study will take place 42 to 1 day prior to administration of the first dose of study drug. Participants will be assessed for their eligibility to participate in the study and must sign an informed consent form prior to performing any invasive study related procedures. The participants will have two visits during the Screening Period.

The following information will be gathered, and screening assessments will be performed for each participant.

- Kidney biopsy - at the Screening Period (after all eligibility assessments have been completed and patient eligibility has been confirmed)

Initial Visit (Day -42 to -7)

- Collect Inform Consent Form
- Demographics
- Medical history (including Alport Syndrome history and historical lab values of proteinuria, creatinine, hematuria up to 5 years back)
- Review of inclusion/exclusion criteria
- Height
- Weight and BMI
- Vital signs
- Detailed physical examination
- 12-lead ECG
- Confirmatory testing of nonsense mutation of Col4A5 in a male or nonsense mutation of Col4A4 or Col4A3 (male or female)
- Blood safety lab tests
- HIV, HBsAg, HCV Ab
- Serum FSH and LH (in female participants 12 to 30 years of age)
- Blood test for pregnancy (only for females 12 to 30 years of age)
- Urinalysis and urine pellet
- Urine for proteinuria (UPCR)
- Urine test for drugs of abuse
- Audiometry assessment
- Tympanometry
- Speech reception threshold
- Otoscopy
- Dizziness and Tinnitus status
- Adverse event monitoring
- Assessment of concomitant medications

Baseline Visit (Day -7 to -1)

Visit should be performed within 7 days prior to dosing and at least 2 weeks after the Audiology assessments were performed at the Initial Visit.

- Urinalysis (only urine pellet)

- Urine for proteinuria (UPCR)
- Audiometry assessment
- Tympanometry
- Speech reception threshold
- Otoscopy
- Dizziness and Tinnitus status
- Adverse event monitoring
- Assessment of concomitant medications

7.1.2 Treatment Period

The following series of procedures will be done during the Treatment Period. If there is a break in treatment of >7 days, the participant will need restart a new 60-day Treatment Period and all of the procedures scheduled on Day 1 will have to be performed.

Day 1

Prior to dosing:

- Reconfirmation of inclusion/exclusion criteria
- Weight and BMI
- Vital signs (up to 90 minutes before dosing)
- Brief physical examination
- Development Stage (pediatric participants only)
- Blood for safety lab tests
- Blood collection for PK (up to 90 minutes before dosing)
- Urinalysis (no urine pellet)
- Urine pregnancy test (only for female 12 to 30 years of age)
- Urine for proteinuria (UPCR)
- Adverse event monitoring
- Assessment of concomitant medications
- Injection administration training

Clinic dosing

Post-dosing

- Vital signs (1 hrs ± 15 min)
- Blood collection for PK (1 hrs ± 15 min)
- Evaluation of injection site (1 hrs ± 15 min)
- Dispense study drug

Days 15, 30, 45 and 60

Prior to dosing:

- Weight, body mass index (on Day 60 only)
- Vital signs (up to 90 min before dosing)

- Brief physical examination
- Development Stage (pediatric participants on Day 60 only)
- Kidney biopsy (on Day 60 ± 3 days)
- Blood safety lab tests
- Blood collection for PK (up to 90 minutes before dosing)
- Urinalysis (including urine pellet on Days 30 and 60 only)
- Urine pregnancy test (only for females 12 to 30 years of age)
- Urine for proteinuria (UPCR)
- Evaluation of injection site
- Adverse event monitoring
- Assessment of concomitant medications
- Return unused study drug

Clinic dosing (self-administration, participant will be observed during injection of ELX-02, and re-trained if necessary)

Post-dosing

- Vital signs (1 hrs ± 15 min)
- 12-lead ECG (on Day 60 only at 1 hrs ± 15 min)
- Blood collection for PK (1 hrs ± 15 min)
- Audiometry assessment (on Days 30 and 60 only)
- Otoscopy (on Days 30 and 60 only)
- Dizziness and Tinnitus status (on Days 30 and 60 only)
- Evaluation of injection site (1 hrs ±15 min)
- Adverse event monitoring
- Dispense study drug (on Days 15, 30 and 45 only)

7.1.3 Safety Follow-up (beginning 4 weeks following the last dose)

For participants who completed the Treatment Period, safety follow up visits will take place on Days 90, 120 and 150. For participants who terminated early, a safety follow-up visit, Day 150, will take place 90 days after last day of study drug administration.

- Blood for safety lab tests
- Urinalysis (no urine pellet)
- Urine for proteinuria (UPCR)
- Audiometry assessment
- Otoscopy
- THI and DHI questionnaires
- Adverse event monitoring
- Assessment of concomitant medications

7.1.4 Unscheduled Visits

An unscheduled visit may take place at any time during the study at the participant'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 medications 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.

7.1.5 Participant Withdrawal

If a participant's participation in the study is prematurely terminated for any reason or participant 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 after dosing has taken place, all efforts should be made to invite the participant to complete at least one safety follow-up visit.

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

If the participant withdraws from the study treatment 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.

7.1.6 Physical Examination

A complete physical examination will be performed at Screening. On other visits, a brief physical examination, focusing on CV, pulmonary, and hearing, will be performed prior to dosing or at any time if the participant is not being dosed on that day. Clinically significant changes from the baseline examination at Screening will be recorded as AEs.

7.1.7 Vital Signs

Vital signs (supine BP, heart rate [HR], respiratory rate [RR], and oral/tympanic temperature) will be measured in supine position after at least 5 minutes of supine rest at the time points specified in Table 1.

On days when vital signs parameters need to be assessed and blood samples need to be collected, the vital signs will be performed before blood sampling.

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

7.1.8 ECGs

The following values will be collected: QT, QTcF, HR, QRS, PR and RR at the time points specified in Table 1.

Any ECG abnormality compared to screening 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.

7.1.9 Developmental Stage

Participants younger than 18 years old will be evaluated for developmental stages based on Tanner stages.[33]

7.1.10 Safety Blood Test

Safety laboratory evaluations will be performed at the timepoints specified in (Table 1). Processing and storage of the samples are detailed in the laboratory manual provided by the Sponsor.

The safety laboratory assessment variables that will be analyzed are outlined in Table 2.

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 Phosphatase (ALP)	Blood urea nitrogen
Albumin	Gamma glutamyl transferase (GGT)	Glucose	Serum Creatinine
Total Bilirubin	Lactate dehydrogenase (LDH)	Sodium	Glomerular filtration rate (GFR*, only at Initial visit)
Alanine aminotransferase (ALT)	Creatine Phosphokinase	Potassium	Magnesium
Phosphate	Calcium		
Hormones			
LH		FSH	
Blood pregnancy test			
Serology			
HIV Ab	HBsAg	HCV Ab	

*Based on CKD-EPI for ages ≥18 and Schwartz formula for participants <18

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

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 is considered stable. Abnormal laboratory test results that are considered to be clinically significant by the Investigator will be reported as an AE.

7.1.11 Blood Sampling for Pharmacokinetic Assessments

Blood samples for determination of ELX-02 plasma concentrations will be taken at the timepoints specified in Table 1.

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 source and missed samples/timepoints confirmed in the eCRF.

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.

7.1.11.1 Additional Blood Tests

7.1.11.1.1 Screening Phase

Blood samples will also be drawn at Screening for confirmation of nonsense mutation Alport Syndrome, and antibodies for HIV, HBsAg, HCV.

7.1.11.1.2 Pregnancy Testing

For female participants of childbearing potential (see definition in Section 6.3), age 12 years and older, serum pregnancy test will be performed at the timepoint specified in Table 1. Pregnancy test for female participants <12 years of age, will be conducted if investigator judges to be necessary.

A negative pregnancy result is required before the participant may receive study drug. Pregnancy tests will also be done whenever 1 menstrual cycle is missed during the active treatment period or when potential pregnancy is otherwise suspected. Pregnancy tests may also be repeated as per request of IRBs/ IECs or if required by local regulations.

7.1.12 Urine Tests

Urine laboratory evaluations will be performed at the timepoints specified in (Table 1). Processing and storage of the samples are detailed in the laboratory manual provided by the Sponsor.

7.1.13 Audiology and Vestibular Assessments

Detailed information on Audiology and Vestibular Assessments will be provided on the Audiology and Vestibular manual.

Participants will have audiometry assessments at Screening Period, Days 30 and 60 (Treatment Period), as well as Days 90, 120 and 150 (Safety Follow-up Period).

During the Screening Period, two sets of audiology assessments will be performed to address Exclusion Criterion #17, designated as Initial and Baseline:

- Initial assessments should be performed within days -42 to-7.
- Baseline assessments should be performed within 7 days prior to dosing and at least 2 weeks after Initial assessments.

7.1.13.1 Audiometric Testing

Participants will be scheduled for audiometric tests conducted by an ENT specialized department and will include PTA and HFA with frequencies up to 16 kHz. Bone conduction should be performed during the Initial and Baseline visits, and must be completed on other visits only if there is a threshold shift (≥15 dB) from Baseline by air conduction frequencies of 4 kHz or lower.

In any case of audiometric significant change (see criteria) from Baseline to days 30, 60, 90, 120, 150, a re-test must be performed after 15 min or up to 24 hours (see section 8.15). The re-test results should be entered into the EDC, and all audiology worksheets required to be shared with the Sponsor.

7.1.13.2 Tympanometry

The measurement of sound reflection from the tympanic membrane as changes in air pressure occur. This procedure should be performed before the Audiometric testing during the Initial and Baseline visits, and must be repeated on other visits only if there is any suspicion of a threshold shift or hearing loss on audiometry (≥ 15 dB at any frequency), or if requested by the Investigator.

7.1.13.3 Otoscopy

A visual inspection of the inner structures of the ear using the otoscope. This test is performed by a qualified physician or audiologist prior to any hearing testing.

7.1.13.4 Speech Reception Threshold

Speech reception threshold (SRT) is the softest intensity sound at which a participant correctly repeats spondee words (bisyllabic words with equal emphasis on both syllables) at least 50% of the time. SRT is to be performed on Initial and Baseline visits, and must be repeated on other visits only if there is any suspicion of a threshold shift or hearing loss on audiometry (≥ 15 dB at any frequency), or if requested by the Investigator.

7.1.13.5 Dizziness and Tinnitus Status

For adults, the status will be evaluated by THI and DHI questionnaires, a self-assessment scale. These questionnaires will be completed just prior to each audiological assessment in the study. Pediatric participants will be questioned by the site staff about their tinnitus/dizziness condition, following by Pediatric DHI if dizziness status is active. Additional information is described on the Audiometry and Vestibular Manual.

7.1.14 Injection Site Reactions

Injection sites will be assessed using the US NIH Division of AIDS (DAIDS) criteria (Table 3). on days when the medication is administered at the clinic. Local reaction will be assessed at the timepoints specified in Table 1.

Table 3: DAIDS Grading Scale Site Reactions to Injection

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 hrs of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hrs treatment	Generalized itching causing inability to perform usual social & functional activities	NA

7.1.15 Kidney Biopsy

The tissue of participants (mandatory for adult participants and optional for pediatric participants) will be evaluated for Col IV expression and kidney structural changes.

Upon participants' consent, participants that performed kidney biopsy in the last 5 years, will be asked to provide the biopsy sample for further analysis to evaluate collagen IV expression.

7.1.15.1 Future Use of Stored Specimens

Pending participant's consent, storage and use of left-over kidney biopsy samples collected as part of study assessments, will be performed for future research related to Alport Syndrome. The left-over biopsy samples will be maintained for up to 5 years.

Pending participant's consent, storage and use of historically collected kidney biopsy will be maintained for up to 5 years.

Genetic testing will not be performed on these samples.

7.1.16 Adverse Events

Adverse events will be collected continuously starting from signing the informed consent form (ICF) until EOS visit for enrolled participants.

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 participant's files.

Common Terminology Criteria for Adverse Events (CTCAC Version 5) will be used to determine the AE safety profile of ELX-02. For more information refer to Section 8.

7.1.17 Concomitant Medications

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

8 ADVERSE EVENT REPORTING

8.1 Adverse Events

All observed or volunteered AEs regardless of treatment group with 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, and may consider participant's medical history, the temporal relationship of drug administration to the event, possible adverse events attributed to the drug class and/or a biologically plausible mechanism when assessing causality (Section 8.9). 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 participant provides informed consent, which is obtained prior to the participant's enrollment in the study, i.e., prior to undergoing any study-related procedure and/or receiving investigational product, through and including 90 calendar days after the last administration of the investigational product. SAEs occurring to a participant 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. On an ad hoc basis the safety committee will review potentially related serious adverse events leading to discontinuation as they are reported.

8.3 Definition of an Adverse Event

An AE is any untoward medical occurrence in a clinical investigation participant administered a product or medical device; the event does not necessarily need 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 Medication Errors

Medication errors may result, in this study, from the administration or consumption of the wrong product, by the wrong participant, at the wrong time, in the wrong way (eg, into muscle) 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 participant 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 participant

Whether or not the medication error is accompanied by an AE, as determined by the Investigator, the medication error is captured as an AE in the eCRF and, if applicable, any associated AE(s) are also captured as AEs in the case report form (CRF).

8.5 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.

8.6 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 participant 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.

8.7 Hospitalization

Hospitalization is defined as any initial admission (even less than 24 hrs) 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 (e.g., 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 (e.g., caregiver relief)
- Skilled nursing facilities
- Nursing homes
- Same-day surgeries (as outpatient/same-day/ambulatory procedures)

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.

8.8 Severity Assessment

Severity assessment will be performed per categories specified in the CTCAE for all AEs except injection site reactions (ISR) which will be classified and graded as per DAIDS guidelines at protocol-defined timepoints. Injection site reactions reported by the participant or assessed outside of defined DAIDS timepoints should be recorded as AEs.

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living

- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

8.9 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. The Investigator may consider the participant's medical history, the temporal relationship of study drug administration to the event, possible adverse events attributed to the drug class and/or a biologically plausible mechanism when assessing causality. 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 World Health Organization (WHO)-Uppsala Monitoring Center (UMC) system for standardized case causality assessment:

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

Certain

- Event or laboratory test abnormality with plausible time relationship to study 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 study 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 study 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 study 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 study drug
- It could readily have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant
- It does not follow a known pattern of response to the study drug

8.10 Action Taken Regarding the Study Drugs

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

- Permanently discontinued
- Stopped temporarily
- Dose reduced
- No action taken
- Unknown/not applicable

8.11 Outcome

The outcome of each AE must be rated as follows (Table 4):

Table 4: Rating Scale for the Outcome of Each Adverse Event

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

8.12 Unexpected Adverse Event

An unexpected AE is any AE which is not listed in the IB or is not listed at the specificity or severity that has been observed for an unapproved study 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 study drug.

8.13 Adverse Events of Special Interest

For this study, adverse events of special interest (AESI) include the following:

a. Nephrotoxicity – graded based on CTCAE. Further criteria are described in Section 8.14.

b. Ototoxicity – Further criteria are described in Section 8.15.

An AESI report must be completed and sent via fax or email within 24 hrs of Investigator's knowledge of the event to the study monitor and Sponsor's representative. In case an AESI is classified as SAE, only the SAE report will be completed and SAE reporting procedures and timelines would be applicable.

8.14 Criteria for Nephrotoxicity

Grading of any nephrotoxic event will be based on CTCAE Grade, which is assessed as degree of elevation of serum creatinine compared to pre-dosing baseline. For stopping dosing in an individual participant based on nephrotoxicity, criteria noted in Section 3.1.1 must be met.

8.15 Criteria for Ototoxicity

8.15.1 Pure Tone Audiometry including High Frequency Audiometry

For PTA (frequencies up to 8000 Hz), CTCAE criteria will be used to define ototoxicity. Any event meeting the criteria for CTCAE Grade 1 or more will be evaluated for potential ototoxicity.

For HFA (frequencies 10,000-16,000 Hz), a threshold shift from baseline of >25 dB averaged at 3 contiguous test frequencies in at least one ear, will be considered potential ototoxicity.

For severity grading of all AEs relating to ototoxicity (including HFA changes), the criteria for AE severity grading based on clinical symptoms as described in Sections 8.6 and 8.8 will be used.

For 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 threshold shifts in PTA and HFA are worse by the following criteria:

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

Participants serve as their own controls for hearing change; if ASHA significant change criteria are met in at least one ear, PTA including HFA should be re-tested 15 minutes later or up to 24 hours (preferably immediately) to confirm the results and all worksheets should be submitted to the Sponsor. In addition to the above criteria being met in at least one ear to suspect ototoxicity, there should be no indication of middle ear abnormality. If ototoxicity is considered, or findings are unclear, especially if middle ear abnormalities are suspected, the Investigator should discuss the results with medical monitor immediately and inform the Sponsor. The Sponsor will consult with the study Auditory and Vestibular expert(s).

Note that above are criteria for defining AE of potential ototoxicity but not the criteria to stop dosing. For an individual participant, stopping criteria noted in Section 3.1 must be met.

Further details are provided in an Auditory and Vestibular Manual.

8.15.2 Tympanometry

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

8.15.3 Speech Reception Threshold

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

8.15.4 Tinnitus Status

If an adult participant has a post-dosing score showing a 5 point increase in THI score from baseline accompanied by a shift in category (e.g., from mild to moderate handicap), the event will be discussed between the Investigator and the medical monitor to determine if further auditory testing will be required. In a pediatric participant, any change of tinnitus status from baseline, will trigger a review by the Sponsor and the Auditory and Vestibular expert(s). Any findings consistent with drug-induced toxicity leading to tinnitus or exacerbation of pre-existing tinnitus on formal evaluation will be re-evaluated 3 months after initial examination and, if persistent, will be deemed permanent.

8.15.5 Dizziness Status

If an adult participant has a post-dosing score showing a 5 point increase in DHI score from baseline accompanied by a shift in category (e.g., from mild to moderate handicap), the event will be discussed between the Investigator and the medical monitor to determine if further vestibular testing will be required. In a pediatric participant, any change of dizziness status from baseline, will trigger a review by the Sponsor and the Auditory and Vestibular expert(s). Any findings consistent with drug-induced vestibular toxicity on formal evaluation will be re-evaluated 3 months after initial examination and, if persistent, will be deemed permanent.

8.16 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 (e.g., 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 (e.g., a nurse reports that she is pregnant and has been exposed to chemotherapeutic products).
3. A male has been exposed (e.g., 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 participant or study participant's partner becomes or is found to be pregnant during the study participant's treatment with the investigational product, the Investigator must submit this information to the CRO on a pregnancy form A. In addition, the Investigator must submit information regarding environmental exposure product in a pregnant woman (e.g., a participant

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 hrs 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 pre-procedure 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 (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.

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 EDP may be requested by the Investigator. Further follow-up of birth outcomes will be handled on a case-by-case basis (e.g., follow-up on preterm infants to identify developmental delays). In the case of paternal exposure, the Investigator will provide the study participant with the Pregnant Partner Release of Information Form to deliver to his partner. The Investigator must document in the source documents that the participant was given the Pregnant Partner Release of Information Form to provide to his partner.

8.17 Follow-up of Adverse Events

Participants 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 study 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 participant has attended the last expected safety follow-up visit and the end of study (EOS visit). If additional follow-up of an AE is warranted, it will be documented in the participant's source data but will not be part of the eCRF. The PI is required to report on any significant findings to the Sponsor.

8.17.1 Data Safety Monitoring Board (DSMB)

DSMB will be comprised of group of independent experts. The DSMB objectives and operational details will be defined in a separate document (DSMB Charter). In this study, once the first 2 participants (age 12-18 years old) complete 4 weeks of treatment, the DSMB will review safety data for the 2 participants and recommend continuation of the study (including possible enrollment of pediatric participants 6-11 years old) or discontinuation due to safety concerns. Outside of this planned meeting, the DSMB will also review potentially related serious adverse events (SAEs) leading to discontinuation or study stoppage as they are reported.

8.18 Occupational Exposure

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

An occupational exposure must be reported to the study drug safety unit within 24 hrs, using the SAE report form, regardless of whether there is an associated AE/SAE. A copy of the completed SAE report form is maintained in the Investigator site file. Since the information does not pertain to a participant enrolled in the study, the information is not entered into the eCRF.

8.19 Withdrawal Due to Adverse Events

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

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

8.20 Eliciting Adverse Event Information

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

8.21 Recording of Adverse Events

All (S)AEs occurring during the clinical investigation must be documented in the source documents and eCRF.

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 eCRF]. 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 participant, 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.

8.22 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.

8.22.1 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 24 hrs 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 participants 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 eCRF 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 participant'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 hrs of Investigator awareness of the event by the CRO.

As noted in Section 8.6, 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 hrs of Investigator awareness. The SRC will review potentially related SAEs leading to discontinuation as they occur.

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 (e.g., if an outpatient study participant initially seeks treatment elsewhere), the Investigator is to report the event within 24 hrs after learning of it and document the time of his or her first awareness of the AE.

8.22.2 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 part of the eCRF. 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.

8.22.3 Sponsor's Reporting Requirements to Regulatory Authorities

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

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

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

After completion 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 participants 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 and General Statistical Methods

No formal sample size or power calculation is performed. The study sample size is based on empirical considerations rather than statistical determination.

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

For categorical data, summary tabulations of the number and percentage of participants within each category of the parameter will be presented. For continuous data, the number of participants, mean, median, standard deviation (SD), minimum, interquartile range (Q1 and Q3), and maximum will be presented.

9.2 Pharmacokinetic Analysis

PK analyses will be based on the PK Population defined as those treated patients who have analyzable PK data without relevant deviation interfering with the PK evaluations.

No formal calculation of pharmacokinetic parameters will be performed. Plasma ELX-02 concentration data will be summarized with descriptive statistics (mean, standard deviation, minimum, median, maximum, geometric mean, N).

9.3 Safety Analysis

Safety evaluation will be based on the Safety population defined as all treated participants who received at least one dose of study medication, including participants prematurely withdrawn from the study.

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

Summary tables using descriptive statistics will be provided for changes from baseline in vital signs, electrocardiograms and safety laboratory tests. Shift tables will be presented for safety laboratory tests.

Summary tables will be generated for PTA, tympanometry, and THI and DHI questionnaires. The degree of hearing loss and any abnormalities detected in ENT examination will be summarized by time point.

Changes in hematuria will be recorded as a categorial variable. Proteinuria will be evaluated as UPCR. The baseline value will be the geometric mean of the screening and pre-Day 1 dosing.

9.4 Efficacy Analysis

Efficacy analyses will be based on the Efficacy Population defined as those participants who have a valid baseline (before dosing) efficacy sample and at least 1 non-missing post-baseline value for an efficacy sample.

Efficacy data will be summarized using descriptive statistics and/or graphical displays.

Efficacy parameters will be described as changes from baseline in PD measurements during Treatment Period. The relationship between ELX-02 and response variables (safety and efficacy) will be explored graphically and, if applicable, using regression models.

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/IEC. All correspondence with the IRB/IEC should be retained in the Investigator file. Copies of IRB/IEC 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 participants. 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 participants. Special attention should be paid to studies that may include vulnerable participants.

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 participants)
- Sponsor-approved participant recruiting materials
- IB (or equivalent information) and addenda
- Available safety information
- Information on compensation for study-related injuries or payment to patients 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 participants
- 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 participants, 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 participants
- New or revised participant recruiting materials approved by the Sponsor
- Revisions to compensation for study-related injuries or payment to participants 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 investigational medicinal product (IMP)
- New information that may adversely affect the safety of the participants or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the participants
- Report of death of any participants under the Investigator's care
- Notification if a new Investigator is responsible for the study
- 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 participants, 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 participants. If a deviation from or a change to the protocol was implemented to eliminate an immediate hazard to study participants, 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 EOS (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 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 Participant Information and Consent/Assent

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

When study data are compiled for transfer to Eloxx Pharmaceuticals and other authorized parties, participant names, addresses, and other identifiable data will be replaced by a numerical code based on a numbering convention in order to de-identify study participants. The study site will maintain

a confidential list of participants who participated in the study, linking each participant'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 participants' personal data consistent with applicable privacy laws.

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

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

Potential participants will be fully informed of the nature of the study and of the risks and requirements of the study before any invasive study-related assessment will be carried out. Each participant at the age of consent (per local requirements) must sign and date a study-specific ICF. Participants not of age of consent must assent, if applicable per local requirements, to participate in the study, and the participant's parent or legal guardian must sign and date a study specific ICF. During the study, participants 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 participants or a parent/ legal guardian of a participant child, 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.

Finally, they will be told that the Investigator will maintain a participant 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 participant to the extent permitted by the applicable law(s) or regulations. By signing the ICF/Assent, the participant is authorizing such access and agrees to allow his or her study physician to recontact the participant 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/Assent, should be non-technical and practical and should be understandable to the participant (or the participant's legally acceptable representative). The participant or parent/legal guardian 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 participant's personally dated signature. After having obtained consent, a copy of the ICF must be given to the participant.

The collection and processing of personal data from participants 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 participants confidential.

The informed consent obtained from the participants includes explicit consent for the processing of personal data and for the Investigator to allow direct access to participants' 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 Participant Recruitment

Advertisements approved by IRBs/IECs 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 participants 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 (i.e., 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 participants against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the Investigator becomes aware of.

10.6 Measures related to COVID-19

As described in Section 1.1, the early onset and rapid progression to renal failure in AS patients with nonsense mutations makes developing therapies in this pre-adolescent and adolescent patients urgent. Despite the high unmet need, there is no approved treatment for AS, therefore this study is required, even during the pandemic.

In order to accommodate study conduct and procedures to the ongoing COVID-19 pandemic situation, the following measures will be taken:

- Enrollment, participant travel and visits are to be performed according to local country and institution regulations, as well as site guidelines

- A study participant who tests positive for COVID-19 is not eligible to participate in the study until the participant is considered recovered, per local country regulations.

- Monitoring visits should be performed according to local country and site guidelines and are permitted to be performed remotely, excluding remote source data verification (SDV)

- Dose interruption in case of COVID-19 occurrence:

    - COVID-19 should be reported as any other Adverse Event
    - The Investigator should perform a full AE assessment and decide on the required action to be taken regarding the study drug, as described in Section 8.10 of the protocol

- If in-clinic study visits during the Treatment and Safety Follow Up Periods (except for Day 1 visit) are not allowed due to regional lockdown or individual participant quarantine:

- Site visits will be performed at home. The study drug will be shipped to the participant and UPCR will be performed at a central lab.
- Audiology assessments will be performed once site visits are resumed.

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 Participants, 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 Participant Identification, Enrollment, and Screening Logs

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

The participant identification and enrollment log will be treated as confidential and will be filed by the Investigator in the study file. All reports and communications related to the study will identify Participants by initials and/or assigned number only.

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

11.3 Study Termination

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the IRB/IEC, 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 participants 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.

11.4 End of Trial

End of trial is defined as last participant in all participating countries, completing all Treatment Period study assessments.

12 QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor or its designee will conduct periodic monitoring visits during study conduct to ensure that the protocol and study requirements as well as GCP are being followed. The monitor will review source documents to confirm that the data recorded on CRFs are accurate (refer to Section 13 for details on how data are to be recorded). The Investigator and institution will allow the Sponsor monitor/auditors or its designee and appropriate regulatory authorities, direct access to source documents. Monitoring visits and/or audits may also occur after study completion.

During study conduct and/or after study completion, the study site may be subject to review by the IEC, and/or to quality assurance audits performed by the Sponsor, or companies working with or on behalf of Eloxx Pharmaceuticals, and/or to inspection by relevant regulatory authorities.

The Investigator or designee will notify the Sponsor, and/or its designee, immediately of any regulatory inspection notification in relation to the study. Furthermore, the Investigator will cooperate with the Sponsor and/or its designee to prepare the study site for the inspection and will allow the Sponsor and/or its designee, whenever feasible, to be present during the inspection. The Investigator will promptly provide copies of the inspection findings to the Sponsor and/or its designee. Before submitting a response to the regulatory authorities, the Investigator will provide the Sponsor or its agents with an opportunity to review and comment on responses to any such findings.

It is important that the Investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

13 DATA HANDLING AND RECORD KEEPING

13.1 Case Report Forms

Electronic data capture (EDC) and information management systems (CTMS for protocol deviations, if applicable) will be used for this study. Clinical data will be entered into the EDC system by site staff. Laboratory data from external vendors will be reported directly to the sites and received via external data transfers to be processed into SAS datasets by the Sponsor/designee, although site staff may be asked to enter select laboratory data directly into the EDC system. Study documents identifying which data are electronic source and which are paper-based will be created.

The Investigator will ensure that the data collected are accurate, complete, and legible. Data within the EDC system will be monitored by the study monitor, who has read-only access to review entered data, during monitoring visits to the clinical site. Following the ICH GCP guidelines, direct access to source documentation (medical records) by the study monitor must be allowed. Any changes required following monitoring will be made by site personnel or the Investigator. Changes made in the EDC system will be documented with a full audit trail within the system.

It is recommended that the author of an entry in the source documents is identifiable.

13.2 Record Retention

To enable evaluations and/or audits from regulatory authorities or the Sponsor, the Investigator agrees to keep records, including the identity of all participants (sufficient information to link records, e.g., CRFs, study source documentation, and hospital records), all originally signed ICFs, safety reporting forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, and telephone call reports). The records should be retained by the Investigator according to the ICH guidelines, according to local regulations, or as specified in the clinical study agreement (CSA), whichever is longer.

If the Investigator becomes unable for any reason to continue to retain study records for the required period (e.g., retirement or relocation), the Sponsor should be prospectively notified. The study records must be transferred to a designee acceptable to the Sponsor, such as another Investigator, another institution, or an independent third party arranged by the Sponsor. Study records must be kept for a minimum of 15 years after completion or discontinuation of the study or for longer if required by applicable local regulations.

The Investigator must obtain the Sponsor's written permission before disposing of any records, even if retention requirements have been met. If required to prolong the retention time, this will be subject to an agreement with the clinical center.

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note	*CFTR* allele variant classification is based on cellular phenotype. Some *CFTR* variants may be cross-classified or lack classification altogether. Therefore, additional variants may be eligible for the study that are not be listed here. Please refer questions to the sponsor.