

# Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses of ELX-02, a Potential Treatment for Genetic Disorders Caused by Nonsense Mutations, in Healthy Volunteers

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## Abstract

ELX-02 is an investigational synthetic eukaryotic ribosome-selective glycoside optimized as a translational read-through molecule that induces read through of nonsense mutations, resulting in normally localized full-length functional proteins. ELX-02 is being developed as a therapy for genetic diseases caused by nonsense mutations. Two phase Ia, randomized, double-blind placebo-controlled, single-ascending-dose studies were conducted in healthy human subjects to evaluate the safety and pharmacokinetics of ELX-02. Single subcutaneously injected doses of ELX-02 between 0.3 mg/kg and 7.5 mg/kg showed an acceptable safety profile without severe or serious drug-related adverse events, including a lack of renal and ototoxicity events. Injection of ELX-02 resulted in a rapid time to peak concentration and elimination phase, with complete elimination from the vascular compartment within 10 hours. ELX-02 area under the concentration-time curve to infinity showed dose-exposure linearity (24-fold increase for a 25-fold dose increase), and the maximum concentration showed dose proportionality (17-fold increase for a 25-fold increase). The mean apparent volume of distribution was dose dependent, suggesting an increased distribution and tissue uptake of ELX-02 at higher doses. The primary route of excretion was in the urine, with the majority of the compound excreted unchanged. These results support the evaluation of the safety, pharmacokinetics, and efficacy of repeat dosing in future studies.

## Keywords

ELX-02, safety, pharmacokinetics, single ascending dose, phase I, healthy volunteers, genetic disorders, nonsense mutations, aminoglycoside, translational readthrough

Premature stop codons are the result of “nonsense” mutations, lead to truncated or absent proteins, and cause a significant number of genetic diseases<sup>1</sup> including cystic fibrosis, cystinosis, Usher syndrome, primary ciliary dyskinesia, mucopolysaccharidosis I, and a variety of cancers.<sup>2</sup> Nonsense mutations are a result of mutations within germline or somatic DNA, inaccurate or inefficient pre-mRNA splicing, or improper RNA editing. According to the human gene mutation database, 12% of all mutations are the result of single point mutations, which lead to premature stop codons.<sup>3</sup> The disease phenotypes caused by nonsense mutations are frequently more severe than those caused by missense mutations because premature stop mutations often result in a complete loss of protein function.<sup>4</sup> Curative therapies

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do not exist for most diseases caused by nonsense mutations, with disease management based primarily on prevention and treatment of complications. In some cases supportive care is all that is currently available.

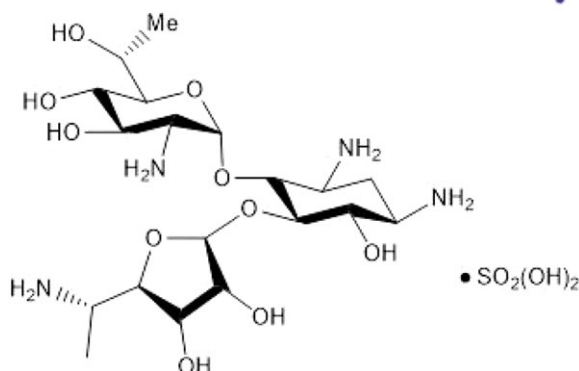
Aminoglycosides are capable of inducing translational read through by binding to the decoding site in the small subunit of the ribosome RNA<sup>5</sup> that normally monitors proper codon-anticodon interactions. In limited short-term studies traditional aminoglycosides have shown the potential to cause translational read through across premature termination codons that results in functional protein and demonstrated clinical efficacy.<sup>6,7</sup> Despite these promising results, traditional aminoglycosides have limited read-through effects and cannot be used as long-term therapies because they can induce nephro- and ototoxicity.<sup>8,9</sup> In addition, chronic use can lead to the development of resistant strains of pathogenic bacteria.<sup>10–12</sup>

ELX-02 is quickly absorbed after subcutaneous (SC) administration and displays dose-proportional exposures with no gender differences, no accumulation in plasma, and is partitioned preferentially to plasma. The plasma protein binding varies across species with essentially no binding in mice and up to 11% in rats and humans; plasma protein binding in dogs was approximately 2%. Tissue distribution studies indicated that the highest concentrations of parent material were found in the kidney and spleen. ELX-02 is not metabolized, being excreted in the urine essentially unchanged as parent material.

In preclinical studies *in vitro*, ELX-02 had no effect on induction or inhibition of cytochrome P450 enzymes or uridine diphosphate glucuronosyltransferase activity, no effect on P-glycoprotein, uptake transporters, or breast cancer resistance protein and displayed low permeability in the Caco-2 assay (data not shown).

ELX-02 [6'-(R)-methyl-5-O-(5-amino-5,6-dideoxy- $\alpha$ -L-talofuranosyl)-paromamine sulfate, also known as NB124<sup>13</sup>] is an investigational, advanced synthetic eukaryotic ribosome-specific glycoside that was designed to improve read through, eliminate antibiotic activity, and minimize the toxicities associated with aminoglycosides<sup>6,7</sup> (Figure 1). It behaves as a translational read through-inducing agent<sup>14</sup> to promote the generation of normally localized, full-length functional proteins from genes containing nonsense mutations and may modulate mRNA stability for affected genes.

Nonclinical studies in cystic fibrosis models bearing loss-of-function nonsense mutations showed that ELX-02 could restore expression and function of the disease-associated genes by inducing translation read through.<sup>15</sup> In addition, ELX-02 increased mRNA from the *CTNS* (lysosomal cystine transporter) gene associated with cystinosis, suggesting that the compound may also modulate nonsense-mediated mRNA decay.<sup>16</sup>



**Figure 1.** Structure of ELX-02.

ELX-02 exerts its read-through activity by increasing the probability that a near-cognate transfer RNA (tRNA) will bind to premature stop codons and displace class 1 releasing factors, thus enabling continuation of translation beyond the premature stop codon and generation of a full-length functional protein.<sup>17–19</sup> At a molecular level, ELX-02 stabilizes the “exo” conformation of 2 conserved adenosine residues in the ribosomal RNA caused by binding of cognate tRNAs and, as a consequence, increases the affinity of the ribosomal A-site for near-cognate tRNAs.<sup>13,20</sup> This phenomenon allows the ribosome to recognize the first 2 bases in the nonsense codon and incorporate a near-cognate amino acid into the nascent polypeptide chain, allowing translational read through of premature stop codons.

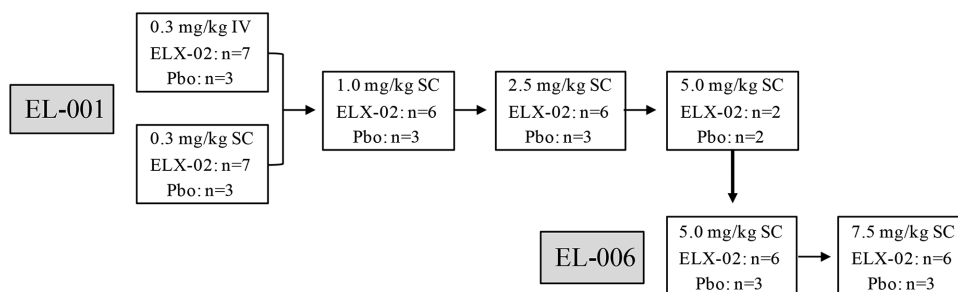
When compared to the aminoglycoside antibiotic gentamicin, ELX-02 demonstrates increased read-through activity attributed to its higher selectivity toward the cytoplasmic ribosome, a 100-fold lower antibacterial activity, and a potentially enhanced safety profile. Recent work<sup>21</sup> has demonstrated that the high affinity of aminoglycosides for mitochondrial versus cytoplasmic ribosomes is a key determinant of toxicity, and ELX-02 has approximately 100-fold lower affinity for the mitochondrial ribosome than gentamicin.<sup>15</sup>

Two phase 1a, randomized, double-blind placebo-controlled, single-dose dose-escalating studies in healthy human subjects were conducted to evaluate the safety and pharmacokinetics (PK) of single SC injected doses of ELX-02 between 0.3 mg/kg and 7.5 mg/kg, as well as an intravenous (IV) dose of 0.3 mg/kg. The combined data are presented here.

## Methods

### Study Conduct and Ethical Considerations

EL-001 (NCT02807961) was conducted at Tel Aviv Sourasky Medical Center, Clinical Research Center (Tel Aviv 6423916, Israel), and EL-006 (NCT03292302)



**Figure 2.** Flowchart of study protocols. Pbo indicates placebo; IV, intravenous; SC, subcutaneous.

was conducted at SGS Life Sciences Services, Clinical Pharmacology Unit (Antwerp, Belgium). The studies were conducted in compliance with the Declaration of Helsinki and with the International Conference on Harmonisation Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95), including the archiving of essential documents. Local institutional review boards (study 001, Tel-Aviv Sourasky Medical Center Helsinki Committee, Tel-Aviv Yafor, Israel; study 006, Commissie Voor Medische Ethiek, ZNA Institutional Review Board, ZNA/OCMW, Antwerp, Belgium) reviewed and approved the study protocols and the informed consent documents. All subjects provided written informed consent before any study-related procedures were performed.

### Study Design

Studies EL-001 and EL-006 were phase 1, randomized, double-blind placebo-controlled, single-dose, dose-escalating studies in healthy male and female subjects. The primary objectives of these studies were to assess the safety and tolerability of single ascending doses of ELX-02 administered SC or IV and to assess the PK of single doses of ELX-02 administered either IV or SC. The secondary objectives of these studies were to determine if a maximum tolerated dose could be attained within the given dose range, to assess the bioavailability of SC administration compared to IV administration, and to assess the linearity and proportionality between ascending SC doses and PK parameters.

Subjects were randomized in a 2:1 ratio to ELX-02 or placebo treatment. Five ELX-02 doses were assessed in the combined studies: 0.3 mg/kg, 1.0 mg/kg, 2.5 mg/kg, 5.0 mg/kg, and 7.5 mg/kg (Figure 2). The 0.3 mg/kg dose was assessed with both IV and SC formulations to determine absolute bioavailability.

Both studies were divided in a screening period from 35 (EL-001) or 42 (EL-006) days before dosing to 2 days before dosing, a treatment period from 1 day before dosing to approximately 3 days after dosing, and an end-of-study visit that took place 7–11 days after dosing. On day 1, in the morning, each subject received a 500-mL normal saline infusion over 60–90 minutes within 120 minutes before dosing to assure proper hy-

dration. This infusion was followed by a single dose of ELX-02 or placebo. Subjects underwent PK assessments (blood and urine) as well as safety assessments (ie, adverse events [AEs], clinical laboratory tests, ECG). Specialized safety parameters, particularly those associated with known toxicities of aminoglycosides to identify any potential early safety signals, were also evaluated. In addition, samples to assess early markers of proximal renal tubular injury (clusterin and kidney injury molecule-1 [KIM-1]) as well as samples to assess the potential read through of housekeeping proteins (cystatin C,  $\beta_2$ -microglobulin, argonaute 1, malate dehydrogenase 1) were collected.

For both studies the decision to progress to the next cohort and to escalate the dose was dependent on the recommendations made by an independent dedicated Data Safety Monitoring Board (DSMB) specific for each study, which evaluated the safety and tolerability of the completed dose cohort.

### Selection of Doses in the Studies

The starting dose in the EL-001 study (0.3 mg/kg) was 1/185th and 1/161st of the human equivalent dose from the no observable adverse effect level (NOAEL) in the dog. The NOAEL in the dog single-ascending-dose toxicology study was equivalent to 56 mg/kg in human, providing a single-dose safety margin of >7-fold for the maximum study dose of 7.5 mg/kg and >10-fold for the anticipated phase 2 dose range of 1.0–5.0 mg/kg.

In the 28-day repeated-dose rat and dog toxicology studies, the exposures at the highest dose without adverse renal findings were 3- to 4-fold higher than the anticipated pharmacologically active dose of 2.5 mg/kg. The exposures at the lowest observable adverse effect level for renal findings were 6- to 9-fold higher than the dose of 2.5 mg/kg in humans. The highest exposure at which no ototoxicity findings were observed was 14-fold higher than the anticipated pharmacologically active dose of 2.5 mg/kg. These data suggest that renal toxicity will be seen before ototoxicity is noted.

Because doses of 5 mg/kg of ELX-02 in the EL-006 study came within a 2-fold safety margin (when compared to the highest dose without renal findings of 28-day repeated-dose studies in rats and dogs) and

within 20- to 35-fold (when compared to the NOAEL of the single-dose study in dogs), patients were monitored carefully for any possible AEs, especially renal toxicity and ototoxicity events.

### Subjects

Eligibility criteria included healthy male and female subjects aged 18 to 45 years with a body mass index (BMI) of 19 to 30 kg/m<sup>2</sup>, total body weight >50 kg and <100 kg, normal renal function (glomerular filtration rate >60 mL/min, based on creatinine plasma concentrations and the Modification of Diet in Renal Disease equation for glomerular filtration rate), no history of hearing loss and no use of any medication with potential to impair renal function (eg, nonsteroidal anti-inflammatory drugs [NSAIDs]) or with ototoxic potential (eg, quinine, salicylates, aminoglycosides) were eligible for the study. Appropriate use of contraception was required for participation in this study for both male and female subjects.

Exclusion criteria included evidence or history of clinically relevant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease, history of ear disease or surgeries, persistent dizziness or persistent tinnitus, vestibular pathology, conductive hearing loss, balance problem, abnormalities in audiometry results at screening, presence of mitochondrial mutations making subject susceptible to aminoglycoside toxicity (EL-006 study only), and abnormal electronystamography/videonystamography or abnormal video/computerized Head Impulse Test (EL-001 study only).

### Treatments

The active ingredient, ELX-02, was a synthetic glycoside manufactured by Aptuit (Oxford) Ltd (Oxfordshire, UK) in compliance with Good Manufacturing Practice. The drug product was manufactured by Albany Molecular Research Ltd (Glasgow, UK) in compliance with Good Manufacturing Practice. The drug was supplied at a concentration of 200 mg/mL. Each vial contained 160 mg of ELX-02 and 0.8 mL of water for injection. The placebo was a standard 0.9% NaCl sterile solution for injection, which was provided by the site pharmacy. The same 0.9% NaCl solution was used to prepare the appropriate dilutions of ELX-02.

### PK Assessments

Blood samples (5 mL) for determination of ELX-02 plasma concentrations were collected using tubes containing tripotassium ethylenediaminetetraacetic acid at the following time points:

- IV administration (30 minutes): In house (days 1–3) predose and at 0.08, 0.25, 0.5, 1, 3, 6, 12,

24, 36, 48, and 144 hours postdose. The PK sampling times were relative to the start of the infusion for the IV arm.

- SC administration: In house (days 1–3) on predose and at 0.25, 0.5, 1, 3, 6, 12, 24, 36, 48, and 144 hours postdose.

Urine samples, to measure the rate of ELX-02 excretion, were collected at the following time points:

- Day 1: predose (first void in the morning)
- Day 1 (0–12 hours postdose): for the first 12 hours subjects were encouraged to void as frequently as possible (ie, asked every hour if they can collect urine), and each individual sample was weighed and processed separately.
- Day 1 (12–24 hours postdose): urine was collected during a period of 12 hours (into 1 container in EL-001) or every 6 hours (ie, 12–18, 19–24 into 2 containers in EL-006). In both studies 24 hours was collected separately. All containers were weighed individually, and the weights were recorded.
- Day 2 (24–48 hours postdose): on day 2 urine was collected after the first void until first morning urine on the next day (48 hours postdose in EL-001) or every 12 hours (ie, 24–36 hours and 37–48 hours in EL-006). In both studies 48 hours was collected separately. All containers were weighed individually, and the weights were recorded.
- ELX-02 in human plasma and urine was quantified using a validated liquid high-performance liquid chromatography method with tandem mass spectrometric detection (LC/MS/MS) at Aptuit (Verona, Italy) using [<sup>2</sup>H<sub>3</sub><sup>13</sup>C]-ELX-02 as the internal standard.

ELX-02 in human plasma and urine was quantified using a validated liquid high-performance liquid chromatography method with LC/MS/MS at Aptuit (Verona, Italy) using [2H<sub>3</sub><sup>13</sup>C]-ELX-02 as the internal standard. Chromatographic separation was performed using a Waters (Milford, Massachusetts) Acquity UPLC HSS T3, 1.8 μm 2.1 × 50 mm analytical column. Mobile phase A consisted of 5 mmol/L ammonium formate + 0.1% (v/v) heptafluorobutyric acid; mobile phase B was methanol. Flow rate was 0.4 mL/min, and temperature was set at 25°C. The run time was approximately 1.5 minutes. An Applied Biosystems (Waltham, Massachusetts)/Sciex (Framingham, Massachusetts) API-4000 MS was used for detection, with TurboIonSpray (Sciex) interface at 600°C.

Plasma samples were processed using methanol precipitation; urine samples were processed by dilution in 0.1% heptafluorobutyric acid. The validated



range of the method was 10 to 10 000 ng/mL. The transition mass (precursor ion  $m/z$  to product ion  $m/z$ ) for ELX-02 was 483  $\rightarrow$  163. For ELX-02 in plasma, the within-run precision was <18.6% (lower limit of quantification), between-run precision was <6.8%, and the accuracy was between 82.5% and 99.2%. For ELX-02 in urine, the within-run precision was <10.2% (lower limit of quantification), between-run precision was <5.1%, and the accuracy was between 88.4% and  $\leq$ 103.1%. PK analysis was performed on the PK population (all subjects having valid PK measurements and excluding subjects with major protocol deviations significantly affecting PK) and plasma PK parameters were calculated with Phoenix WinNonlin 6.2 (or later) software (Certara, Princeton, New Jersey) using a non-compartmental analysis method. Urine PK parameters were derived in SAS 9.2 (or later) (SAS Institute, Cary, North Carolina). Actual sampling times were used for PK analysis (when actual sampling times were not available, planned times were used instead).

PK analysis was performed on the PK population (all subjects having valid PK measurements and excluding subjects with major protocol deviations significantly affecting PK), and plasma PK parameters were calculated with WinNonlin Phoenix 6.2 (or later; Certara) software using a noncompartmental analysis method. PK parameters for ELX-02 in plasma included maximal plasma concentration ( $C_{max}$ ), time to maximal plasma concentration ( $t_{max}$ ), area under the plasma concentration-time curve calculated between time of administration and time  $t$  ( $AUC_t$ ), and elimination half-life ( $t_{1/2}$ ). Dose-normalized PK parameters were also calculated ( $C_{max}/dose$  and  $AUC/dose$ ).

Absolute bioavailability was calculated as the ratio between the  $AUC_{inf}$  SC and the  $AUC_{inf}$  IV (100% bioavailability) of ELX-02 0.3 mg/kg using a linear model. The least-squared means of the differences in  $AUC_{inf}$  (log-transformed) between SC and IV administrations and its 90% CI was calculated from the fitted model and then antilog transformed. The least-squares means of  $AUC_{inf}$  and its 95% confidence limits for SC and IV administrations were calculated in a similar manner. Dose linearity was assessed for  $C_{max}$  and  $AUC_{inf}$  in the SC cohorts using a linear model.

Urine PK parameters were derived in SAS 9.2 (or later). Actual sampling times were used for PK analysis (when actual sampling times were not available, planned times were used instead). For urine PK, the amount of drug excreted in urine ( $A_e$ ) during a specified collection interval was calculated as:  $A_e = \sum C_{ur} \times V_{ur}$ , where  $C_{ur}$  is the concentration in urine in the specified collection interval and  $V_{ur}$  is the volume of urine in the specified collection. PK parameters for ELX-02 in urine included  $A_{e0-1h}$ ,  $A_{e1-2h}$ ,  $A_{e2-3h}$ , etc ... until  $A_{e11-12h}$ ,  $A_{e12-18h}$ ,  $A_{e18-24h}$ ,  $A_{e24-36h}$ , and  $A_{e36-48h}$ .

Cumulative  $A_e$  (cumulative urinary excretion from administration to a chosen hour postdose) was also calculated, along with fraction of dose excreted unchanged ( $F_e$ ) and renal clearance ( $A_{e0-48h}/AUC_{0-48h}$ ).

### Safety Assessments

The safety analysis included all subjects who were exposed to the study drug either in the EL-001 or in the EL-006 study (either to placebo or to active compound). Safety measures included (1) AEs; (2) physical examinations; (3) vital signs; (4) ECG measurements; (5) clinical laboratory tests; (6) auditory assessments and questionnaires, including otology, tympanometry, speech recognition threshold, pure tone audiometry, high-frequency audiometry, and tinnitus questionnaires; and (7) vestibular questionnaires. AEs of interest were nephrotoxicity (according to changes in serum creatinine), ototoxicity (auditory and vestibular), and drug-related hypersensitivity reactions (including injection site reactions). The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4.03) was used to determine the AE safety profile of ELX-02. The causality of AEs was determined by the principal investigator. Renal injury biomarkers (KIM-1 and clusterin) and read through of housekeeping genes were assessed as exploratory laboratory end points but not used in the formal evaluation of safety.

A data safety monitoring board (DSMB) in each study reviewed the safety information generated for each cohort's subjects on their completion of all assessments. Each DSMB included independent physicians specialized in relevant medical fields who monitored general events. In addition, a separate special adjudication committee composed of an audiologist (PhD) and an otorhinolaryngologist physician monitored and assessed events that could be ototoxic in nature. The primary responsibility of each DSMB was to evaluate the safety data collected for all subjects in the recently completed cohort and the safe conduct of the study and provided the Sponsor with a report summarizing the conclusions of the meeting and its recommendation whether to proceed to the next cohort or otherwise. The decision to proceed to a higher dose level was made by the sponsor after consideration of the DSMB's and the principal investigator's recommendations following the safety assessments and based on the dose escalation stopping rules.

### Statistical Analyses

Analyses were conducted on pooled data from the EL-001 and EL-006 studies. No formal hypothesis testing was carried out for these studies. For both studies, no formal sample size was estimated. The planned number of subjects included in each dose group was deemed

**Table 1.** Subject Demographics

Parameter	Placebo (N = 20)	ELX-02 0.3 mg/kg IV (N = 7)	ELX-02 0.3 mg/kg SC (N = 7)	ELX-02 1.0 mg/kg SC (N = 6)	ELX-02 2.5 mg/kg SC (N = 6)	ELX-02 5.0 mg/kg SC (N = 8)	ELX-02 7.5 mg/kg SC (N = 6)	All ELX-02 Treatments (N = 40)	All Subjects (N = 60)
Sex, n (%)									
Female	7 (35.0)	1 (14.3)	1 (14.3)	1 (16.7)	0	3 (37.5)	4 (66.7)	10 (25.0)	17 (28.3)
Male	13 (65.0)	6 (85.7)	6 (85.7)	5 (83.3)	6 (100)	5 (62.5)	2 (33.3)	30 (75.0)	43 (71.7)
Race, n (%)									
White	20 (100)	7 (100)	7 (100)	6 (100)	6 (100)	8 (100)	6 (100)	40 (100)	60 (100)
Age, median (range), y	27.5 (20–39)	27.0 (21–29)	21.0 (19–23)	22.0 (18–27)	20.5 (19–25)	31.0 (21–40)	29.0 (26–34)	24.5 (18–40)	26.0 (18–40)
BMI, median (range) kg/m <sup>2</sup>	23.95 (20.1–30.0)	23.60 (20.6–26.6)	22.90 (20.9–29.9)	24.15 (19.7–26.5)	24.20 (20.5–28.8)	24.35 (20.1–27.5)	24.30 (21.0–27.3)	23.75 (19.7–29.9)	23.85 (19.7–30.0)

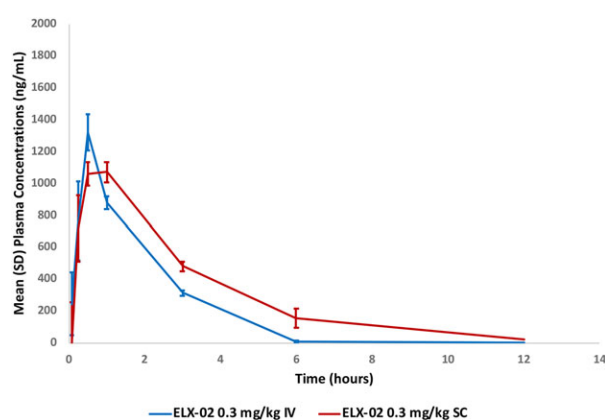
BMI indicates body mass index; IV, intravenous; N, number of subjects with available data; n, number of subjects with that observation; SC, subcutaneous.

sufficient to respond to exploratory safety/tolerability and PK purposes. PK analysis was performed on the PK population, and plasma PK parameters were calculated with WinNonlin Phoenix 6.2 (or later) software using a noncompartmental analysis method. AEs were summarized by number and percentage of subjects experiencing AEs. Continuous safety parameters were summarized per treatment by means of descriptive statistics at each analysis visit and time point, for actual values and for the absolute changes from baseline. Categorical safety results were summarized by frequency, showing the results at each analysis visit and time point.

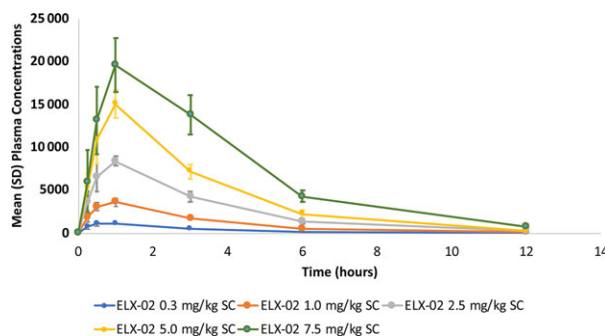
## Results

### Disposition and Demographics

In total, 86 subjects were screened, of whom 60 were randomized (40 to receive ELX-02 and 20 to receive placebo). There were 42 subjects enrolled in Israel and 18 subjects enrolled in Belgium. Of the subjects who received ELX-02, 7 received 0.3 mg/kg ELX-02 IV, 7 received 0.3 mg/kg ELX-02 SC, 6 received 1.0 mg/kg ELX-02 SC, 6 received 2.5 mg/kg ELX-02 SC, 8 received 5.0 mg/kg ELX-02 SC, and 6 received 7.5 mg/kg ELX-02 SC. All subjects completed the studies. The complete disposition of subjects in the pooled studies is shown in Figure 2. Demographic data and baseline characteristics were similar across treatment groups (Table 1). All 60 randomized and treated subjects were white. In total, 17/60 (28.3%) subjects were female with 10/40 (25.0%) subjects in the ELX-02 group and 7/20 (35.0%) in the placebo group. The overall median age was 26.0 (18 to 40) years, with a median age in the ELX-02 group of 24.5 (18 to 40) years and 27.5 (20 to 39) years in the placebo group. The median BMI for all subjects was 23.85 (19.7 to 30.0) kg/m<sup>2</sup> with a median BMI in the ELX-02 group of 23.75 (19.7 to 29.9) kg/m<sup>2</sup> and 23.95 (20.1 to 30.0) kg/m<sup>2</sup> in the placebo group.



**Figure 3.** Mean ( $\pm$  SD) plasma concentration-vs-time profiles for ELX-02 0.3 mg/kg IV and ELX-02 0.3 mg/kg SC. The lower limit of quantification is 10 ng/mL. IV indicates intravenous; SC, subcutaneous.



**Figure 4.** Mean ( $\pm$  SD) ELX-02 plasma concentration-vs-time profiles for ELX-02 0.3 to 7.5 mg/kg SC. The lower limit of quantification is 10 ng/mL. SC indicates subcutaneous.

### Plasma PK

Mean ELX-02 plasma concentration-versus-time profiles are presented in Figure 3 and Figure 4. The key PK parameter data are summarized in Table 2. After a single IV infusion over 0.5 hour at the dose of 0.3 mg/kg, ELX-02 reached  $C_{max}$  rapidly ( $t_{max}$ ), within 0.5 hour after the start of the infusion (Figure 3; Table 2). The

**Table 2.** Summary of ELX-02 Plasma PK Parameters Following a Single IV Dose of 0.3 mg/kg and Single SC Doses of 0.3, 1.0, 2.5, 5.0, and 7.5 mg/kg

PK Parameter (Unit)	ELX-02 0.3 mg/kg IV (N = 7)	ELX-02 0.3 mg/kg SC (N = 7)	ELX-02 1.0 mg/kg SC (N = 6)	ELX-02 2.5 mg/kg SC (N = 6)	ELX-02 5.0 mg/kg SC (N = 8)	ELX-02 7.5 mg/kg SC (N = 6)
C <sub>max</sub> (ng/mL)	1320 ± 246	1144 ± 142	3576 ± 502	8370 ± 548	15 030 ± 1652	19 605 ± 3137
t <sub>max</sub> (h)	0.50 (0.50–0.50)	0.50 (0.50–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)
AUC <sub>0–12h</sub> (ng·h/mL)	3111 ± 413	3891 ± 358	13 131 ± 1729	31 153 ± 2380	52 946 ± 6074	86 891 ± 10 439
AUC <sub>0–24h</sub> (ng·h/mL)	NC (n = 0)	NC (n = 0)	NC (n = 1)	32 468 ± 2543	54 923 ± 6569	91 722 ± 11 332
AUC <sub>0–48h</sub> (ng·h/mL)	NC (n = 0)	NC (n = 0)	NC (n = 0)	NC (n = 0)	NC (n = 0)	94 149 ± 11 842 (n = 5)
AUC <sub>0–inf</sub> (ng·h/mL)	3012 ± 418	3957 ± 373	13 434 ± 1812	32 546 ± 2558	55 521 ± 7126 (n = 7)	95 177 ± 17 336 (n = 3)
t <sub>1/2</sub> (h)	1.75 ± 0.174	2.01 ± 0.155	2.14 ± 0.373	2.86 ± 0.178	3.73 ± 1.08 (n = 7)	8.11 ± 3.19 (n = 3)
CL/(F)* (L/h)	5.73 ± 0.550	5.83 ± 0.752	5.50 ± 0.672	5.70 ± 0.607	6.23 ± 0.715 (n = 7)	5.94 ± 1.22 (n = 3)
Vd/(F)* (L)	11.7 ± 1.47	16.9 ± 2.50	16.9 ± 3.36	23.5 ± 2.19	33.1 ± 8.77 (n = 7)	70.5 ± 33.8 (n = 3)
F <sub>abs</sub>	...	0.98	...	...	...	...

AUC<sub>t</sub> indicates area under the plasma concentration–time curve calculated between time of administration and time t; C<sub>max</sub>, maximal plasma concentration; CL, clearance; F, bioavailability; F<sub>abs</sub>, geometric mean ratio of AUC<sub>0–inf</sub> of 0.3 mg/kg SC vs 0.3 mg/kg IV; IV, intravenous; N, number of subjects; n, number of subjects with available data; NC, not calculated; PK, pharmacokinetics; SC, subcutaneous; t<sub>1/2</sub>, elimination half-life; t<sub>max</sub>, time to maximal plasma concentration; Vd, steady-state volume of distribution.

Values are arithmetic mean ± SD except for t<sub>max</sub> (median [min–max]) and for F<sub>abs</sub>. \*CL and Vd for IV route, CL/F and Vd/F for SC route.

elimination was rapid with a short terminal mean t<sub>1/2</sub> of 1.75 hours. Mean residence time of ELX-02 was about 2 hours, mean volume of distribution was 11.7 L, and mean total body clearance was about 5.7 L/h. The volume of distribution was low, suggesting a distribution restricted to plasma and extracellular fluids.

Comparison of a single administration of 0.3 mg/kg of ELX-02 either as an IV infusion during 0.5 hour or SC injection resulted in a similar t<sub>max</sub> with a median t<sub>max</sub> of 0.5 hour (Figure 3; Table 2). The decline of concentration was monophasic and rapid regardless of the route of administration. Mean C<sub>max</sub> was slightly lower after SC than IV administration, and mean terminal t<sub>1/2</sub> and clearance were comparable (t<sub>1/2</sub> = 1.75 hours for IV and about 2 hours for the SC route), and clearance (apparent for SC) about 5.7–5.8 L/h.

After single SC administrations, ELX-02 was rapidly absorbed with a median t<sub>max</sub> of 0.5 hour for the lowest dose 0.3 mg/kg and 1 hour for the other doses (Figure 4; Table 2). The elimination was rapid, with mean terminal t<sub>1/2</sub> ranging between about 2 and 4 hours for lower doses (0.3 to 5.0 mg/kg), whereas mean t<sub>1/2</sub> was longer for the highest dose (about 8 hours for 7.5 mg/kg) as expected with the use of a noncompartmental model in a situation of multiple-exponential elimination. In the mean plasma concentration–time profiles (Figure 4), the decline of plasma concentrations was monophasic at low doses, whereas it was biphasic for doses of 2.5 and 5.0 mg/kg and multiphasic for the dose of 7.5 mg/kg, with concentrations at higher doses quantifiable for a longer period of time. Mean residence time was about 3–4 hours, and mean apparent body clearance was about 6 L/h for all doses. Mean apparent volume of distribution was dose dependent with values of about 16.9–70.5 L for doses of 0.3–7.5 mg/kg. These data suggest a larger distribution with higher doses in

total body fluids and potentially in tissue. Over the entire dose range (0.3 to 7.5 mg/kg SC), ELX-02 AUC<sub>0–inf</sub> showed dose-exposure linearity (24-fold increase in exposure for a 25-fold dose increase), and C<sub>max</sub> showed a somewhat less than dose proportionality (17-fold increase for a 25-fold increase). In addition, the change in dose from 0.3 to 2.5 mg/kg is 8.3-fold, and mean C<sub>max</sub> increased 7.3-fold. A 3-fold change in dose from 2.5 mg/kg to 7.5 mg/kg resulted in a 2.3-fold increase in this parameter.

### Absolute Bioavailability

The estimated absolute bioavailability was calculated as the ratio of geometric mean area under the plasma concentration–time curve extrapolated to infinity (geometric mean ratio of AUC<sub>0–inf</sub>)/dose of 0.3 mg/kg SC versus 0.3 mg/kg IV (Table 2), and bioavailability was calculated at 0.98 (Table 3). This result suggests that ELX-02 is nearly 100% bioavailable when given SC.

### Urinary Excretion

Plots showing individual cumulative fraction of ELX-02 excreted unchanged in urine (in percentage of dose) versus time of collection (or end of collection interval) are presented in Figure 5. A summary of urinary excretion parameters is presented in Table 3.

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

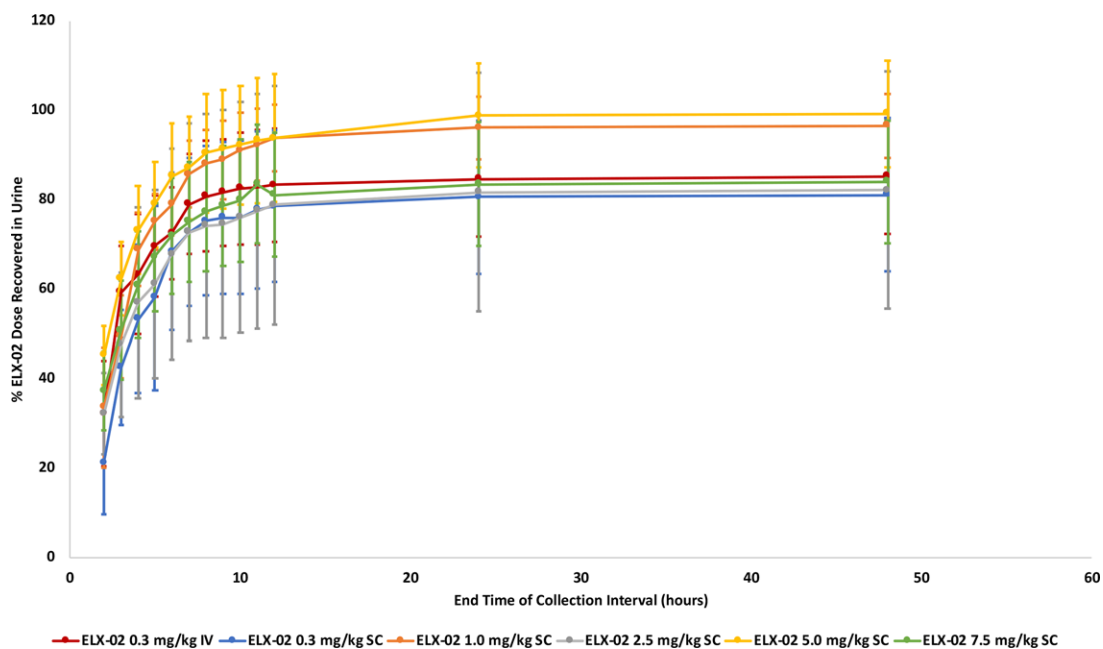
**Table 3.** Summary of ELX-02 Urinary PK Parameters Following Single IV Dose of 0.3 mg/kg and Single SC Dose of 0.3, 1.0, 2.5, 5.0 and 7.5 mg/kg<sup>a</sup>

PK Parameter (Unit)	ELX-02 0.3 mg/kg IV (N = 7)	ELX-02 0.3 mg/kg SC (N = 7)	ELX-02 1.0 mg/kg SC (N = 6)	ELX-02 2.5 mg/kg SC (N = 6)	ELX-02 5.0 mg/kg SC (N = 8)	ELX-02 7.5 mg/kg SC (N = 6)
Ae <sub>0-12h</sub> (mg)	14.8 ± 4.65 (n = 6)	18.1 ± 4.65	68.2 ± 5.29	144 ± 48.0	327 ± 75.5	437 ± 107
Ae <sub>0-24h</sub> (mg)	15.1 ± 4.72 (n = 6)	18.6 ± 4.67	69.9 ± 5.34	149 ± 48.1	345 ± 74.6 (n = 7)	450 ± 111
Ae <sub>0-48h</sub> (mg)	15.2 ± 4.73 (n = 6)	18.7 ± 4.72	70.3 ± 5.42	150 ± 47.9	346 ± 75.4 (n = 7)	453 ± 111
Ae <sub>0-12h</sub> (%dose)	83.3 ± 12.7 (n = 6)	78.5 ± 17.0	93.7 ± 7.47	78.8 ± 26.8	93.8 ± 14.3	81.1 ± 13.9
Ae <sub>0-24h</sub> (%dose)	84.7 ± 13.1 (n = 6)	80.6 ± 17.2	96.1 ± 7.06	81.6 ± 26.7	98.8 ± 11.7 (n = 7)	83.5 ± 13.8
Ae <sub>0-48h</sub> (%dose)	85.2 ± 13.0 (n = 6)	81.1 ± 17.3	96.6 ± 7.14	82.1 ± 26.6	99.2 ± 11.9 (n = 7)	84.0 ± 13.8
CL <sub>r</sub> (L/h)	4.75 ± 1.16 (n = 4)	4.69 ± 1.20	5.25 ± 0.554	4.63 ± 1.48	6.13 ± 0.903	5.14 ± 1.56

Ae indicates amount of drug excreted; CL<sub>r</sub>, relative clearance calculated as Ae<sub>0-12h</sub>/AUC<sub>0-12h</sub>; IV, intravenous; N, number of subjects; n, number of subjects with available data; PK, pharmacokinetic; SC, subcutaneous.

Values are arithmetic means ± SD.

<sup>a</sup>Subject 201 (ELX-02 0.3 mg/kg IV) showed anomalous urinary data for the second sample collected postdose, which biased the estimation of fraction excretion unchanged in urine for this subject. This subject's data were excluded from the descriptive statistics.

**Figure 5.** Mean (±SD) cumulative amount excreted in urine vs collection end time after a single dose of ELX-02 (0.3 mg/kg IV and 0.3 to 7.5 mg/kg SC). IV indicates intravenous; SC, subcutaneous.

### Safety and Tolerability Overview

ELX-02 was shown to be generally well tolerated over the dose range of the single-ascending-dose studies. In total, 25/40 (62.5%) subjects experienced at least 1 treatment-emergent AE (TEAE) after administration of ELX-02 and 9/20 (45.0%) subjects after administration of placebo. All TEAEs after administration of ELX-02 were mild in severity. None of the TEAEs had a significant, serious, or fatal outcome.

The system organ class (SOC) with the highest incidence of subjects reporting TEAEs was General Disorders and Administration Site Conditions with 2/20

(10%) in the placebo group and 12/40 (30%) across the ELX-02 treatment groups, with the highest incidences occurring in the 0.3 mg/kg and 7.5 mg/kg SC groups, 4/7 (57.1%) and 4/6 (66.7%), respectively. The most frequently reported preferred terms for these events were injection-site reaction, injection-site erythema, and injection-site pain. All injection site events were mild in intensity and resolved 15 to 20 minutes postadministration. The second and third most frequently affected SOCs were Ear and Labyrinth Disorders (ear discomfort, ear pain) and Nervous System Disorders (headache, dizziness) (Supplemental Content).



TEAEs considered treatment related by the investigator were reported in 17/40 (42.5%) subjects after administration of ELX-02 and 4/20 (20.0%) subjects after administration of placebo. Injection-site reaction was the most frequent treatment-related TEAE.

Clinical laboratory-related TEAEs (mild) were reported in 1/40 (2.5%) subjects after administration of ELX-02 0.3 mg/kg IV (prothrombin time prolonged) and 1/20 (5.0%) subjects after administration of placebo (thrombocytopenia). The event of prothrombin time prolongation was considered by the investigator to be possibly related to treatment. It was reported 2 days after treatment administration and resolved after 5 days. This finding was not observed at any of the higher doses evaluated. Other laboratory abnormalities were not considered clinically significant. Treatment-emergent abnormalities in vital signs, ECG, and physical examinations were rare and considered not clinically significant.

### *AEs of Special Interest: Nephrotoxicity and Ototoxicity*

AEs related to nephrotoxicity and ototoxicity are of particular interest due to their association with the use of traditional aminoglycosides.<sup>6,7</sup>

Nephrotoxicity was assessed by a change in serum creatinine from baseline at specified time points evaluated according to the CTCAE guidelines. No renal toxicity was observed over the ELX-02 dose range evaluated in the present studies. Serum creatinine levels remained normal at all time points. No TEAEs were reported in any cohort in the SOC of renal and urinary disorders, nor were there any significant changes from baseline to end of treatment between placebo- and ELX-02-treated cohorts in clinical laboratory tests evaluating renal function including serum creatinine.

Auditory-related TEAEs considered to be AEs of special interest were experienced by 4 subjects (1/40 [2.5%] in the ELX-02 0.3 mg/kg IV group, 1/40 [2.5%] in the ELX-02 5.0 mg/kg SC group, and 2/20 [10.0%] in the placebo group). The 2 auditory-related TEAEs in ELX-02 subjects were considered unrelated to ELX-02 by the investigator and the independent expert audiologist.

Ototoxicity was evaluated by auditory and vestibular assessments as well as by questionnaires. Auditory test abnormalities were rare and did not correlate with increasing ELX-02 doses. No abnormalities in questionnaire scores (Dizziness Handicap Inventory, Tinnitus Handicap Inventory) were observed. An independent audiology consultant reviewed all data and did not find the data to be consistent with any reported pattern of aminoglycoside-induced toxicity.

### *Safety-Related Exploratory End Points*

Changes over time in the levels of early markers of renal injury (KIM-1 and clusterin) were used as an early exploratory indicator of proximal tubular injury. No relevant trends were observed in changes from baseline in normalized clusterin or KIM-1 values over time, in line with a lack of nephrotoxicity based on serum creatinine.

The potential of ELX-02 to result in aberrant read through of normal proteins (ie, translation past normal stop codons) was assessed in patient white blood cell samples in EL-001 using the housekeeping proteins cystatin C and  $\beta_2$ -microglobulin and in EL-006 using argonaute 1 and malate dehydrogenase 1 (2 proteins that undergo selective read through in response to environmental signals). The results showed that treatment with ELX-02 up to the highest dose of 7.5 mg/kg did not lead to aberrant read through of any of these proteins. All subjects showed expression of the tested proteins at the expected molecular weights.

### **Discussion**

ELX-02 is an advanced synthetic eukaryotic ribosome-specific aminoglycoside that is being developed as a potential treatment for diseases associated with nonsense mutations. This drug was designed to improve read through, eliminate antibiotic activity, and mitigate the long-term nephrotoxicity and ototoxicity associated with aminoglycosides such as gentamycin.

These single-administration phase 1, first-in-human studies were conducted with doses bracketing the anticipated efficacious dose range of 1.0–5.0 mg/kg. In these studies injection of ELX-02 either IV or SC at the 0.3 mg/kg dose results in a rapidly reached  $C_{max}$  with a  $t_{max}$  for maximum (peak) drug concentration of 0.5 to 1 hour, depending on the route of administration, a rapid elimination phase with a terminal  $t_{1/2}$  of ~2 hours, and complete elimination from the vascular compartment within 10 hours. The absolute bioavailability was calculated at 0.98. Plasma exposure (both  $C_{max}$  and AUC) of ELX-02 increased dose proportionally. Mean apparent volume of distribution was dose-dependent with values of about 16.9–70.5 L for doses of 0.3–7.5 mg/kg. These data suggest a larger distribution with higher doses in total body fluids and potentially in tissue. The primary route of excretion was in the urine, with the majority of the compound excreted unchanged. Recovery of the injected dose in urine was described by a first-order approximation with a small fraction retained in tissues that is excreted in a delayed fashion. Renal clearance estimates are consistent with glomerular filtration rate values in healthy individuals (80–120 mL/min). As expected, ELX-02 PK results are in line with the known PK properties of traditional aminoglycosides. As with many

aminoglycosides, ELX-02 is poorly absorbed orally; when administered parenterally, it achieves rapid  $C_{max}$ , has a low volume of distribution, and is eliminated unchanged via the kidneys.

Overall, ELX-02 was well tolerated and exhibited a favorable safety profile supportive of continued evaluation in repeat-dose human studies. There were no correlations between the incidence, severity, or relatedness to study drug of TEAEs and the ELX-02 dose. All TEAEs resulting from ELX-02 administration were mild with the exception of a single moderate auditory AE of interest but of unclear physiological significance and without clinical impact. None of the TEAEs had a significant, serious, or fatal outcome. The most frequent AEs considered related to ELX-02 by the investigators were injection-site events in 11/40 (27.5%) subjects, comprising “injection-site reaction” in 4 subjects, “injection-site erythema” in 3 subjects, “injection-site pain” in 2 subjects, “injection-site discomfort” in 1 subject, and “injection-site hematoma” in 1 subject. By comparison, 2/20 (10.0%) subjects experienced injection-site events after administration of placebo, suggesting a mild but manageable obstacle for ELX-02 administered by SC injection. In addition, the results showed no evidence of aberrant read-through activity.

A key finding was the lack of renal toxicity and ototoxicity at the ELX-02 doses tested. Previous studies have demonstrated that use of aminoglycosides such as gentamycin may have limited clinical use in the treatment of chronic diseases due to the potential risks of nephrotoxicity and ototoxicity.<sup>21</sup> In the present studies, there was no evidence that injection of ELX-02 had any impact on renal, auditory, or vestibular function in single doses. Serum creatinine levels remained normal, as did biomarkers of early renal tubular injury following injection of ELX-02 either IV or SC at doses of 0.3 to 7.5 mg. These studies, thoroughly assessed auditory function using a battery of tests that included pure-tone audiometry, high-frequency audiometry, tympanometry, and Speech Reception Threshold. The CTCAE and American Speech Language Hearing Association guidelines for ototoxicity were used to evaluate and adjudicate AEs by pure-tone audiometry (normal hearing range) and high-frequency audiometry. The results of these tests showed no evidence of ototoxic effects over the ELX-02 dosage range studied (up to 7.5 mg/kg) and support the further development of ELX-02.

## Conclusions

Although the safety, tolerability, PK, and read-through ability of ELX-02 have been established in nonclinical in vitro and in vivo models, the present studies provide the first confirmatory clinical data in healthy male and

female volunteers. The safety and PK data from these combined studies suggest that ELX-02 administered by SC injection is bioavailable and generally well tolerated, with no significant identified risks, including renal toxicity and ototoxicity, at exposures in the anticipated therapeutic range. These results support the evaluation of the safety and PK of repeat dosing in healthy subjects and safety, PK, and efficacy in patients suffering from genetic diseases caused by nonsense mutations.

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## Declaration of Conflicting Interest and Financial Disclosure

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.