Cystinosis nonsense mutation read-through mediated by ELX-02 restores protein function using in vitro and in vivo models

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Nephropathic Cystinosis is a recessive lysosomal storage disease that leads to Fanconi Syndrome and end stage renal disease

Nephropathic Cystinosis results from defective clearance of cystine from lysosomes leading to the formation of cystine crystals. Early onset (infantile) cystinosis first manifests with Fanconi syndrome (polyuria, polydipsia, loss of electrolytes, etc.), progressive proteinuria and eventual renal insufficiency. Cystinosis accounts for 5% of all childhood cases of kidney failure1. Other organs/tissues also manifest symptoms including: skin, eyes, endocrine, bone and muscle.

Loss of function mutations in the *Cystinosin Lysosomal Cystine Transporter* (*CTNS*) leads to cystinosis

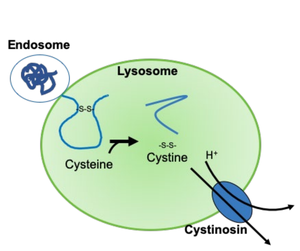

Figure 1: Proteins with disulfide bonds are trafficked via endosomes to the lysosome where cysteine reduces the disulfide bonds, enabling proteolysis and releasing cystine. Normally cystine is quickly exported into the cytosol via cystinosin, a cystine-proton symporter. Loss of cystinosin leads to accumulation of lysosomal cystine, inhibition of proteolysis and oxidative stress.

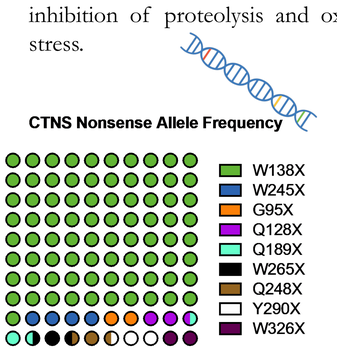

Figure 2: Cystinosis has a worldwide incidence[2] of 1 in 100-200k with regional incidence spikes. A founder nonsense mutation, *W138X* in Québec results in an incidence of 1 in 62,500[3]. *W138X* is the most common nonsense allele for cystinosis, representing 81% of nonsense alleles and 6% of the patient population in Europe, North American and Australia[4].

ELX-02 is a Eukaryotic Ribosomal Selective Glycoside (ERSG) which can rescue full-length protein via ribosomal read-through

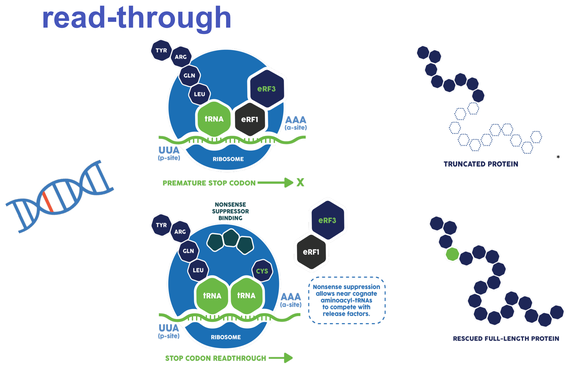

Figure 3: ELX-02 binds the ribosome A-site in a manner that allows near-cognate tRNA incorporation in instances where a point mutation has introduced a premature stop codon. This can result in restoration of essential functional proteins.

Eloxx compounds permit dose-dependent nonsense mutation read-through, measured by protein expression

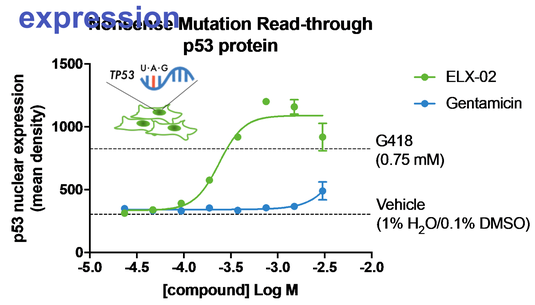

Figure 4: ELX-02 was evaluated for read-through using DMS114 cells, which harbor a native *R213X* nonsense mutation in the *TP53* gene, in a high-throughput immunofluorescence assay measuring p53 protein localized to the nucleus. Error bars represent SD. G418 positive control represents E_{max}.

ELX-02 increases CTNS protein, function and mRNA in patient-derived fibroblasts with the *W138X* allele

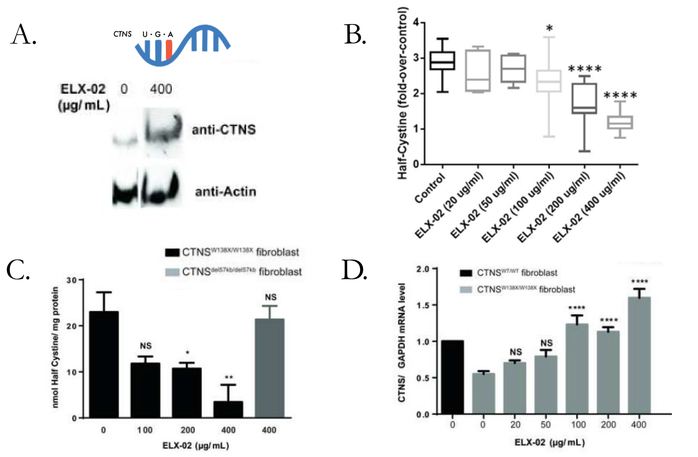

Figure 5: (A) Western blot demonstration of CTNS protein increase in patient-derived fibroblasts bearing homozygous 'UGA' *CTNS W138X* genotype[5]. (B) ELX-02 concentration dependent reduction in fibroblast half-cystine, a measure of CTNS protein function. (C) Read-through mediated CTNS functional activity is dependent on presence of a nonsense allele[5]. (D) Dose-dependent increase in *W138X CTNS* mRNA consistent with interruption of nonsense-mediated mRNA decay with ELX-02[5].

ELX-02 reduces kidney half-cystine accumulation in mouse model of *nonsense-mediated* cystinosis

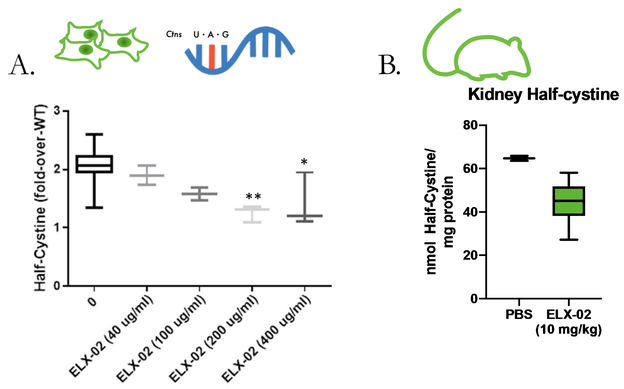

Figure 6: (A) ELX-02 dependent reduction in half-cystine levels are observed in mouse embryonic fibroblasts derived from homozygous *Ctns*^{Y226X} mice. (B) Kidney half-cystine was reduced after 7 days of subcutaneous ELX-02 (10 mg/kg, n=14) administration in homozygous *Ctns*^{Y226X} mice compared to vehicle (PBS, n=2) control.

ELX-02 pharmacokinetic profile demonstrates favorable kidney exposure

plasma

Dose	Day	C_{max} (ng/mL)	T_{max}[a] (min)	$AUC_{0\text{-}t}$[b] (ng.min/mL)	T_{last} (min)	$t_{1/2}$ (min)
9 mg/kg	1	15,100	15	683,000	240	27.14
	22	11,500	15	412,000	120	29.59

a. Median value

b. $AUC_{0\text{-}t}$ = area under the plasma concentration-time curve (AUC) from the start of dosing (0) to the last quantifiable time point (t)

kidney

Dose	Day	C_{max} (ng/g)	T_{max}[a] (min)	$AUC_{0\text{-}t}$[b] (ng.min/g)	T_{last} (min)	$t_{1/2}$ (min)
9 mg/kg	1	45,300	41	9,230,000	480	728.99
	22	30,800	24	5,600,000	480	451.81

a. Median value

b. $AUC_{0\text{-}t}$ = area under the plasma concentration-time curve (AUC) from the start of dosing (0) to the last quantifiable time point (t)

Table 1: A summary of pharmacokinetic parameters for ELX-02 in the plasma and kidney delivered by subcutaneous injection in homozygous *Ctns*^{Y226X} mice.

References

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2) Levy, M. & Feingold, J. Estimating prevalence in single-gene kidney diseases progressing to renal failure. *Kidney Int.* (2000).
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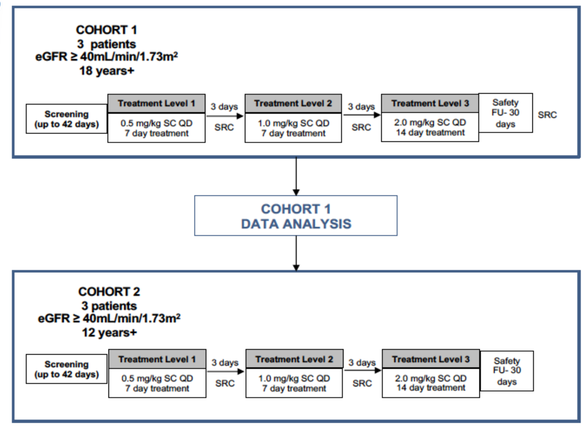
4) CTNS Nonsense Mutation Screen (CyNoMus) Project report, (2019)
5) Goodyer, P. *et al.* The novel aminoglycoside, ELX-02, permits *CTNS*^{W138X} translational read-through and restores lysosomal cystine efflux in cystinosis. PLoS ONE. In Press
6) ClinicalTrials.gov Identifier: NCT04069260, https://clinicaltrials.gov/ct2/show/NCT04069260

A Phase 2 study of ELX-02 in patients with Nephropathic Cystinosis



Primary Endpoints

1. The incidence and characteristics of adverse events (AEs) associated with different dose levels of ELX-02.
2. Blood and urine PK parameters of ELX-02 following the first dose and at steady state at each dose level.

Secondary Endpoints

1. PD as assessed by changes from baseline in WBC cystine levels following ELX-02 treatment.
2. Dose and PK relationship.

Exploratory Endpoints

1. Dose and PD relationship.
2. PK and PD relationship.
3. Changes from baseline in renal injury biomarkers.

Figure 7: Open-label Phase 2 clinical trial design to evaluate the safety, tolerability, PK, and PD of multiple dose levels of daily subcutaneously administered ELX-02 in patients with nonsense-mediated cystinosis. SRC, safety review committee. Nominal doses are presented which may be adjusted for renal function.

Beyond cystinosis, ELX-02 read-through is observed in other nonsense-mediated disease: Autosomal Dominant Polycystic Kidney Disease

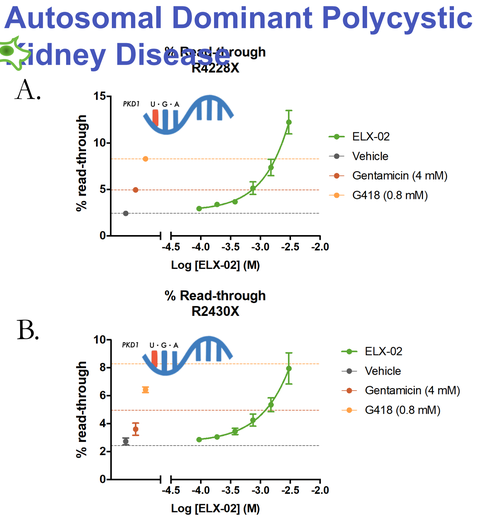


Figure 8: ELX-02 was evaluated against ADPKD nonsense alleles using a plasmid-based, dual-luciferase approach. Briefly, short sequences that include *PKD1* nonsense mutations were cloned into the linker region between Renilla and Firefly luciferase reporters. Plasmids are then transiently transfected into HeLa cells to evaluate compound read-through. This model permits an early assessment of read-through potential against disease-specific mutations. Activity against *R448X PKD1* mutation (A) and *R2430X PKD1* (B) are represented here.

Conclusions

- ✓ ELX-02 read-through is sufficient to produce functional CTNS protein and increase *CTNS* mRNA.
- ✓ Kidney exposure and demonstration of efficacy *in vivo* support dose-range selection for a Phase 2 clinical trial of ELX-02 in Nephropathic Cystinosis.
- ✓ Completion of a Phase 1 study in renal insufficient participants provides modeling necessary for dose adjustments based on renal function.

Acknowledgments

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