Investigational Drug ELX-02 Mediates CFTR Nonsense Mutation Read-through to Increase *CFTR* mRNA, CFTR Protein Translation and CFTR Function

M. Goddeeris[1], J. Mullenders[2], A. Leubitz[1], K. Banks[1], V.M. Mutyam[3], X. Xue[3], S.M. Rowe[3], D. Bedwell[3], T. Baasov[4], G. Williams[1]

[1]Eloxx Pharmaceuticals, Waltham, MA, US; [2]Hubrecht Organoid Technology (HUB), Utrecht, NL; [3]University of Alabama at Birmingham; [4]Technion-Israel Institute of Technology, Haifa, Israel

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

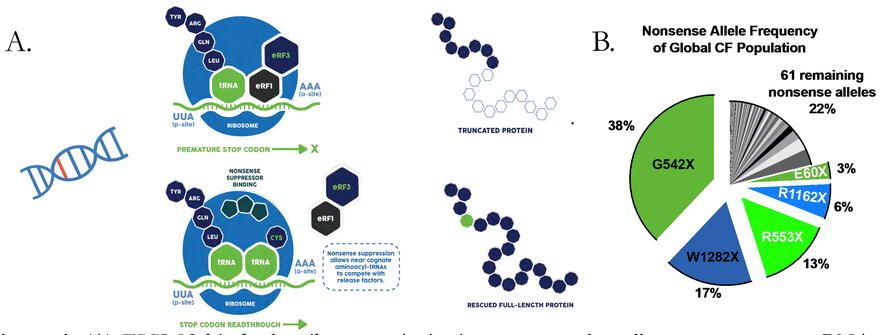

Figure 1: (A) ELX-02 binds the ribosome A-site in a manner that allows near-cognate tRNA incorporation in instances where a point mutation has introduced a premature stop codon. This can result in restoration of essential functional proteins. (B) Global nonsense allele frequency in the cystic fibrosis (CF) population. Curated from *CFTR2* database (*http://cftr2.org*).

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

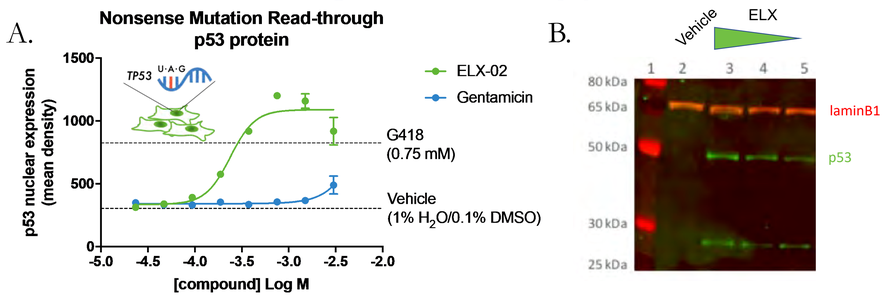

Figure 2: (A) ELX-02 was evaluated for read-through using DMS114 cells, which harbor a native *R213X* nonsense mutation in *TP53*, in a high-throughput immunofluorescence assay measuring p53 protein localized to the nucleus. Error bars represent SD. G418 positive control represents E_{max}. (B) Representative western blot detection of full length p53 after administration of ELX compound. Also refer to Bidou et al. 2017 for additional demonstration of p53 protein restoration by ELX-02.

ELX-02 increases CFTR activity by read-through in *G542X* FRT and HBE cell models

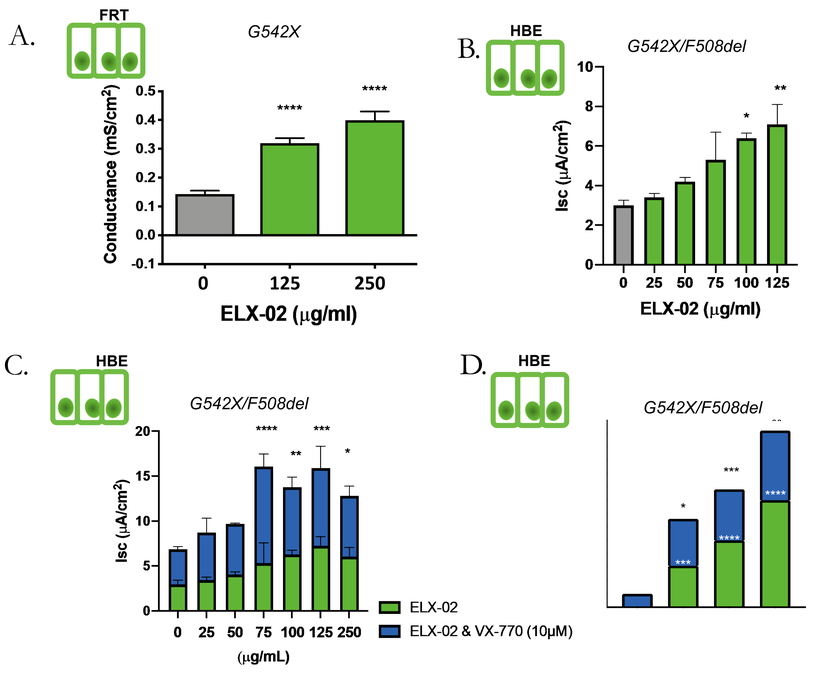

Figure 3: (A) CFTR function was measured in Fisher rat thyroid (FRT) cells overexpressing human *CFTR-G542X* as change from baseline after addition of 10 μM forskolin. ELX-02 exposure was 48 hours. (B) Short circuit current (Isc) was measured in Ussing Chambers following 48 hour ELX-02 or (C) ELX-02 + 10 μM VX-770 in *G542X/F508del* human bronchial epithelial cells. (D) Time-dependence of ELX-02 (125 μg/mL) mediated CFTR activity * p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001. Also refer to Xue et al. 2014.

Reference

1. The Clinical and Functional TRanslation of CFTR (CFTR2); available at http://cftr2.org. ©Copyright 2011 US CF Foundation, Johns Hopkins University, The Hospital for Sick Children
2. Bidou et al. Characterization of new-generation aminoglycoside promoting premature termination codon read-through in cancer cells. *RNA Biology*. 14(3):378-388 (2017).
3. Xue et al., Synthetic aminoglycosides efficiently suppress cystic fibrosis transmembrane conductance regulator nonsense mutations and are enhanced by ivacaftor. *Am J Respir Cell Mol Biol.* 50(4):805-816 (2014).

ELX-02 significantly increases organoid CFTR activity across prevalent CF nonsense alleles and genotypes

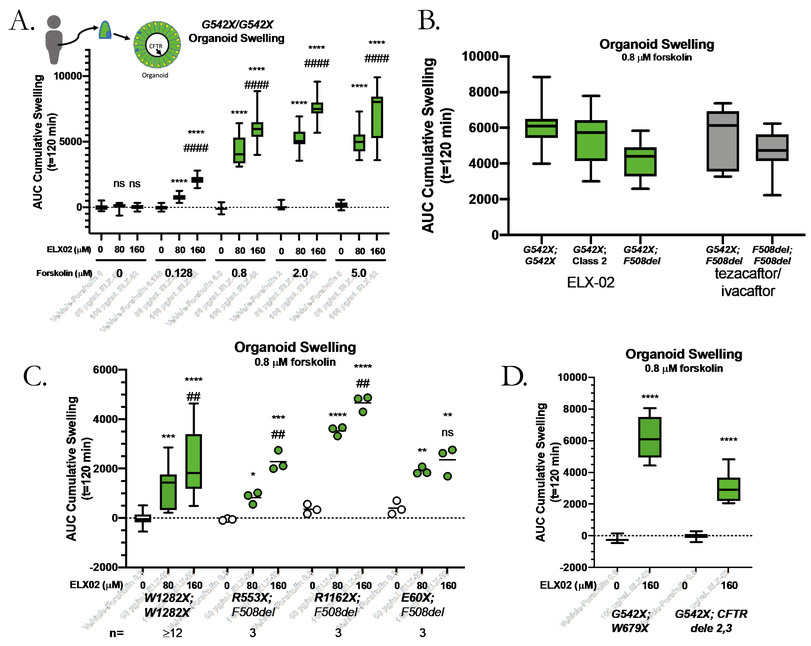

Figure 4: (A) Organoids derived from rectal biopsy stem cells of *G542X/G542X* donor demonstrate dose dependent CFTR-mediated organoid swelling across forskolin induction levels. (B) Organoid swelling with ELX-02 (160 μM) across *G542X* genotype groups in comparison to reference tezacaftor/ivacaftor (3 μM) administration in *F508del* organoids (n=3 subjects) indicates ELX-02 response is consistent with a clinically meaningful response of an approved therapy. (C) ELX-02 mediated CFTR function increases are observed across CF causing nonsense mutations. (D) Investigation into additional genotypes responsive to ELX-02 continue at Eloxx and in collaboration with HIT-CF. * p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001 versus control, # represent comparison to next lower concentration.

ELX-02 increases *CFTR* mRNA stability and increases CFTR protein in *G542X* organoids

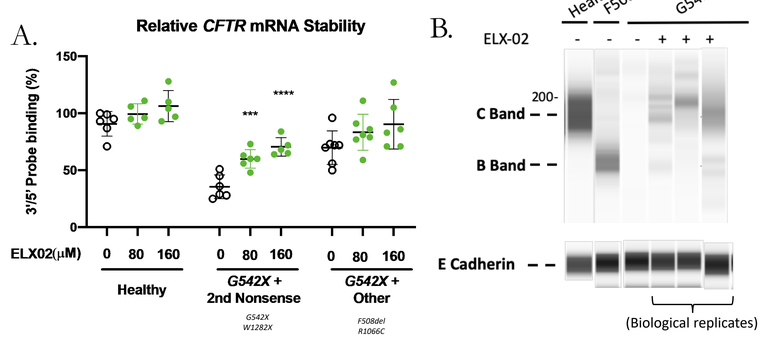

Figure 5: (A) Percentage of 3'/5' mRNA probe detection by Nanostring of CFTR mRNA demonstrated reduced stability of nonsense alleles, likely attributed to nonsense mediated mRNA decay and the increase in stability observed after incubation with ELX-02. ***p<0.001, ****p<0.0001 (B) Capillary native CFTR protein detection (Simple Western, WES) demonstrating C band detection in *G542X* homozygous organoids incubated with ELX-02 (160 μM) for 48 hours. Likewise, ELX-02 treatment demonstrates increased CFTR protein in *G542X* expressing FRT and transgenic mouse models (Xue et al. 2014).

Repeated ELX-02 administration increased CFTR activity in CF *G542X* transgenic mouse model

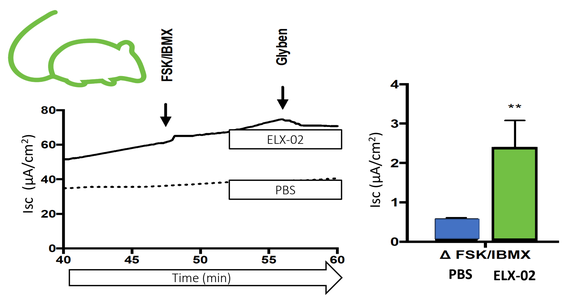

Figure 6: ELX-02 increased CFTR activity in *G542X* mouse model. ELX-02 was administered at 60 mg/kg SC 2x/week for 4-weeks (9x) to n=5 male *Cftr* knockout mice expressing a human *CFTR-G542X* transgene under a intestine-specific fatty acid binding protein promoter. Up to 7 mice were treated with PBS in a similar manner. Ussing chamber (Isc) measurements of intestine tissue (N=4-7 intestine sections/mouse) stimulated with forskolin (10 μM) and IBMX (100 μM). Similar results were obtained with 30 & 60 mg/kg SC/Daily (Xue et al. 2014).

ELX-02 preclinical efficacy-associated exposures translate to selected clinical trial exposure and ascending dose range

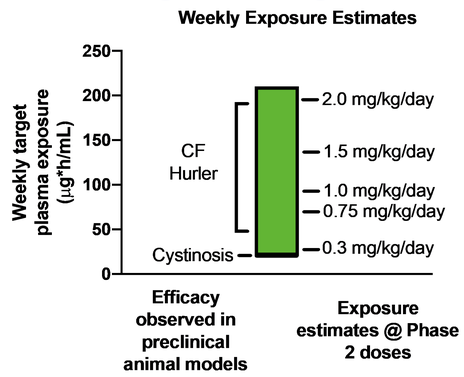

Figure 7: Preclinical mouse studies across disease models have demonstrated a range of efficacious exposures, from 20 μg*hr/mL in a cystinosis model to 47.5-190 μg*hr/mL in CF and Hurler mouse models. Based on SAD and MAD healthy volunteer results, a range of daily doses were selected for our Phase 2 cystic fibrosis clinical trial that match this exposure range.

Conclusions

- ✓ Pronounced ELX-02 mediated CFTR read-through is demonstrated in FRT, transgenic mice, and patient-derived HBE cells and organoids. Significant and meaningful activity is observed against the top 5 most prevalent nonsense alleles, representing >75% of the CF nonsense population. We continue to identify new responsive genotypes.
- ✓ ELX-02 results in a pronounced increase in both CFTR protein expression and mRNA stability further supporting proposed mechanism of action.
- ✓ ELX-02 preclinical efficacy-associated exposures translate to the selected Phase 2 clinical trial ascending dose ranges and exposures.