ELX-02 Pharmacokinetic Profile Appropriate for CF Patient Use

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ELX-02, a novel small molecule translational read-through agent, demonstrates restoration of protein production by enabling the eukaryotic ribosome to read-through nonsense mutations

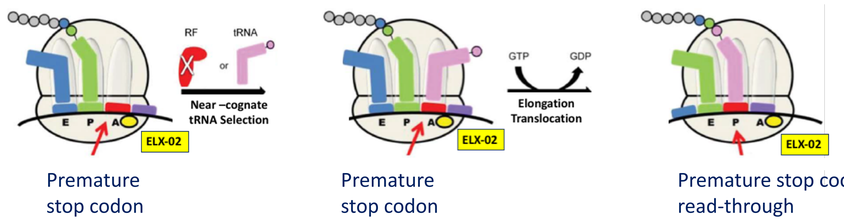


Figure 1: ELX-02 mechanism of action. ELX-02 binds to ribosomal RNA near the site of codon-anticodon recognition in the aminoacyl-tRNA site (A site) stabilizes the tRNA-mRNA interaction in the A site. This results in read-through of nonsense mutations and restores translation of full length proteins.[1-3]

ELX-02 restores CFTR function in organoids and HBE's with nonsense mutations

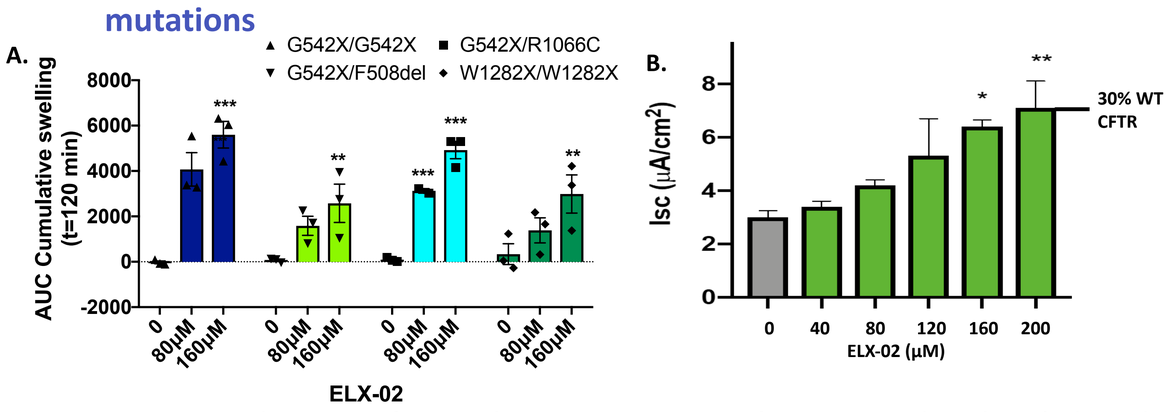
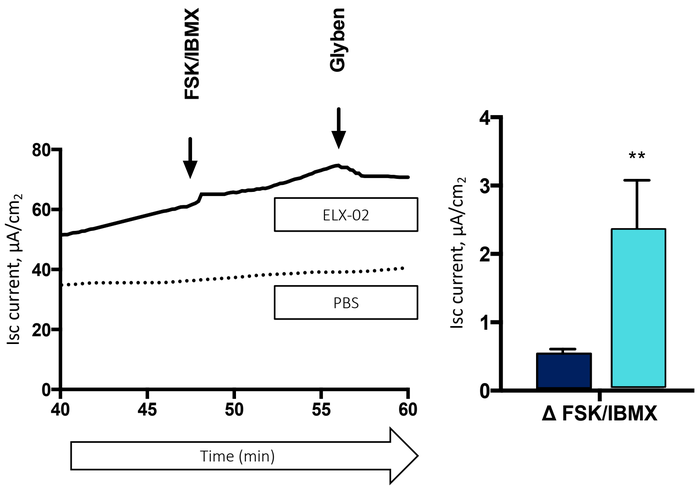


Figure 2: ELX-02 mediates CFTR functional restoration in Cystic Fibrosis organoids and HBE's derived from *G542X* or W1282X nonsense carrier patients. (A) ELX-02 mediates a significant restoration of CFTR function as measured via swelling of the organoids in multiple G542X and W1282X nonsense carrying organoids (0.8µM Forskolin). 3 independent studies each conducted in triplicates. Two way Anova. **p<0.005, ***p<0.001. (B) ELX-02 mediates a significant CFTR current restoration in G542X HBE cells reaching 30% of WT. HBE cells were incubated for 2 days with ELX-02 *p<0.05, **p<0.01 One way Anova. (Internal Study No. CF-01 and 2018 ECFS meeting abstract and presentation)

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

Figure 3: ELX-02 increased CFTR activity in G542X mouse model. ELX-02 given at 60 mg/Kg SC 2x/week for 4-week (9x) to 5 male Cftr knockout mice expressing a human CFTRG542X transgene under intestine-specific rat fatty acid binding protein. Up to 7 mice were treated with PBS in a similar manner. Outcome: Ussing chamber (Isc) measurements of intestine tissue (N=4-7 intestine sections/mouse) stimulated with forskolin (10 µM) and IBMX (100 µM). (Internal Study No. CF-01 and 2018 ECFS meeting abstract and presentation)



Mouse and Rat PK Data Shows Plasma Dose Proportionality and Linearity with Prolonged Tissue Exposure

A. Rat and Mouse Plasma PK

Dose (mg/kg/dose)	Parameters- Rat (M/F combined)		
	C_{max} (ng/mL)	AUC_{last} (ng.h/mL)	$t_{1/2}$ (h)
15	24350	29450	0.73 (M only)
30	49500	62150	0.7
45	81200	100400	0.7 (F only)
Mouse (M)			
10	18393	15162	0.4
30	47348	34269	1.1

B. Mouse Tissue PK

Dose (mg/kg/dose)		Parameters		
		C_{max} (ng/mL)	AUC_t (ng.h/mL)	$t_{1/2}$ (h)
10	Kidney	77179	427419	53
30	Kidney	163926	1212102	44
10	Spleen	327	41124	279
30	Spleen	934	126972	217
10	Lung	123	9012	272
30	Lung	320	22644	76

Figure 4: Rat and Mouse Plasma PK data demonstrate dose proportionality and similar exposures and short plasma t½ with mouse tissue PK showing prolonged exposure. (A). Mean rat (day 180) and mouse (day 14) Cmax, AUC_{last} and $t_{1/2}$ following twice/weekly SC dosing (N=6/sex rat; N=15 mouse). (B). Mean mouse tissue Cmax, AUC_{last} and $t_{1/2}$ following twice/weekly SC dosing for 14 days.

Healthy Volunteer SAD Plasma Exposure and Urine Elimination Data Shows Dose Proportionality and Renal Clearance

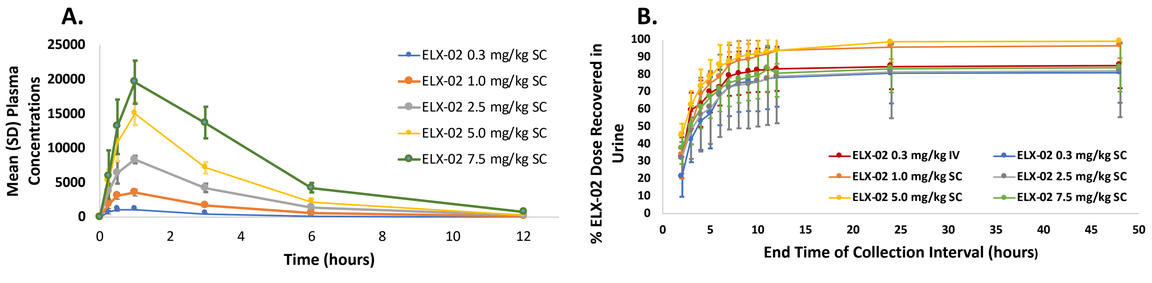


Figure 5: SAD ELX-02 Plasma Concentrations and Urine Elimination values demonstrate dose level proportionality and renal clearance across all dose levels. (A). Mean plasma concentrations (ng/ml) following single SC administration in healthy individuals at doses of 0.3, 1.0, 2.5, 5.0 and 7.5 mg/kg up to 12 hrs post-dose. (B). Percent ELX-02 recovered in urine following single IV/SC administration.[4]

On-going Healthy Volunteer Multiple Ascending Dose (MAD) Study

MAD Study Design

- Randomized, double-blinded, placebo controlled, MD escalating study healthy M/F subjects
- 5 cohorts of 9 subjects/cohort , randomized to received MD of ELX-02 or placebo/2:1 ratio
- 6 subjects received ELX-02 and 3 received placebo, 9 total doses
- Doses were 0.1, 0.3, 1.0 2.5 and 5.0 mg/kg SC twice a week for 28 days (5 mg/kg cohort on-going)

MAD Study Objectives

- Primary
  - Assess the safety and tolerability of multiple ascending SC doses of ELX-02
  - To study the PK of ELX-02 dosed as multiple SC doses
- Secondary
  - Assess whether a MTD is attained within the dose range
  - To assess linearity between ascending SC doses and PK parameters.

Preliminary Healthy Volunteers MAD Plasma Concentrations Show Dose Proportionality and Lack of Accumulation

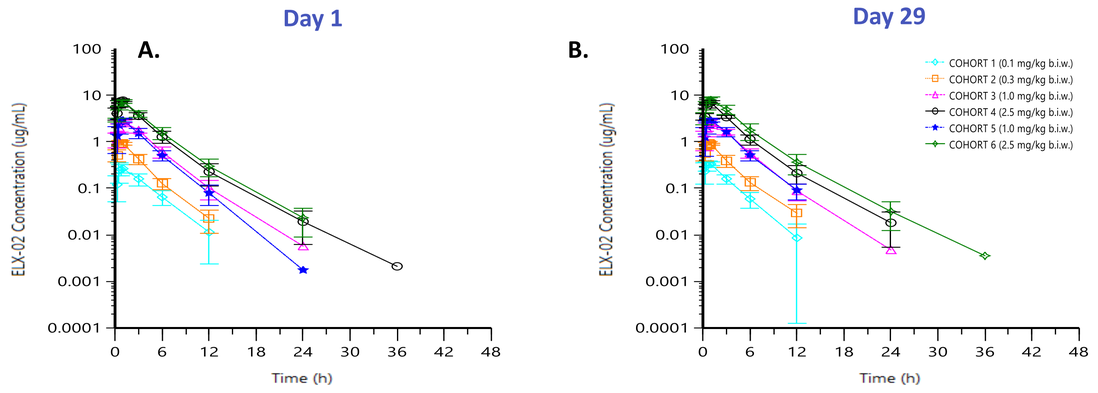


Figure 6: ELX-02 Administered SC twice/week across all dose levels demonstrated dose proportional exposures and lack of accumulation up to day 29. Plasma concentrations measured on day 1-2 (A) and days 29-30 (B) following twice weekly SC administrations of ELX-02 at doses 0.1, 0.3, 1.0 and 2.5 mg/kg. ELX-02 was rapidly absorbed with a median t_{max} of 0.5 hour for the lowest dose of 0.3 mg/kg and of 1 hour for the other doses. Profiles are very similar between both day 1 and day 29.

Preliminary Healthy Volunteer MAD Study C_{max} and AUC_t Exposures Demonstrates Dose Proportionality and Lack of Accumulation

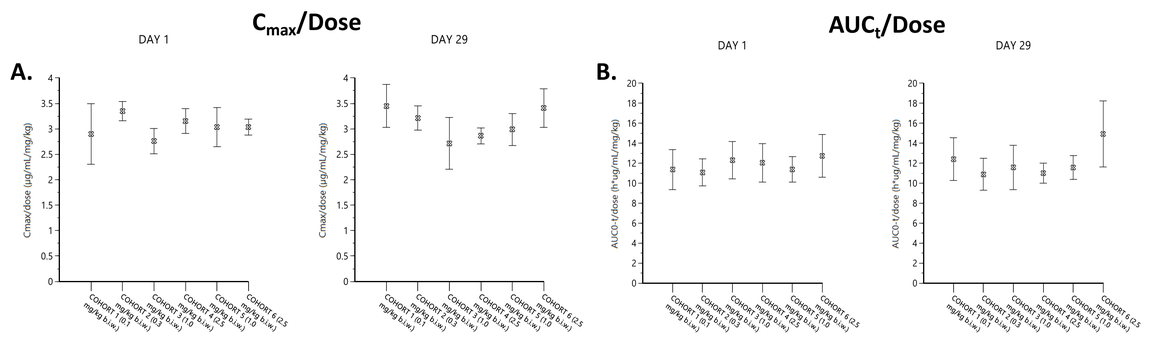


Figure 7: Exposures (C_{max} and AUC_t) demonstrate dose proportionality and lack of accumulation following twice/weekly dosing for up to 28-days in healthy volunteers . (A-B) Dose relative to Cmax and AUC_t values across dose levels on days 1 and 29, respectively.

Physiologically Based PK Modeling - Consistency of Dose and Tissue Data

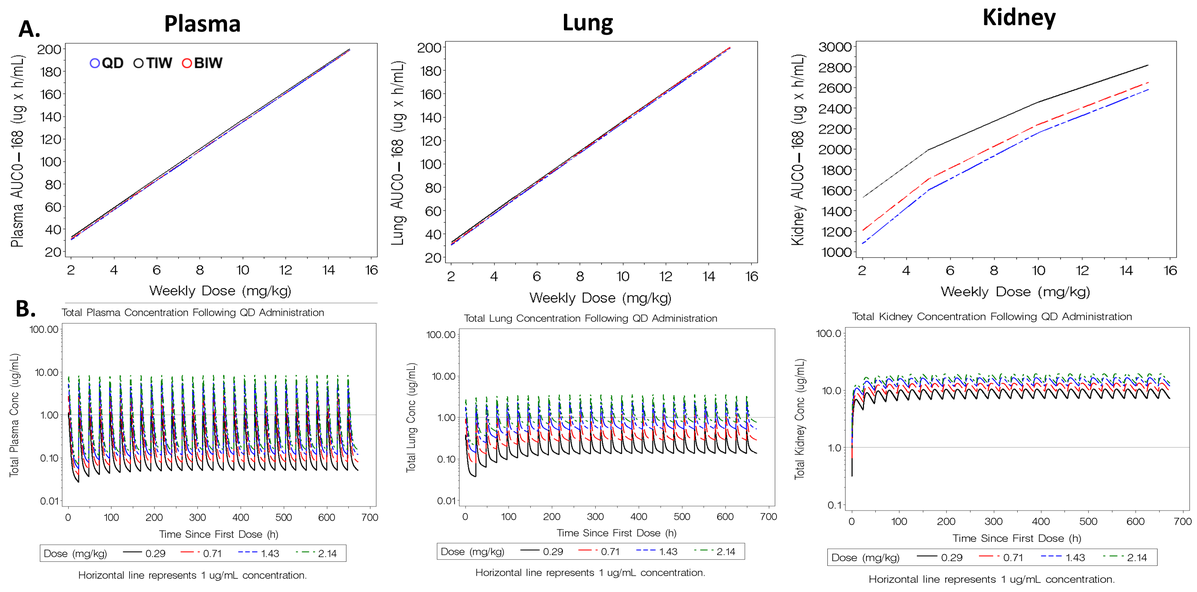


Figure 8: PBPK Modeling shows consistency with both clinical and animal data for plasma exposures across twice/week, daily and three times/week administration. Tissue AUC's show dose proportionality in PBPK modeling. (A). Plasma and Tissue PBPK modeling based on animal plasma/tissue and human plasma data (QD=daily, TIW=3 times/wk and BIW=2 times/wk dosing). (B). Daily Dosing Peak and Tough PBPK modeling for plasma and tissues (Dose of 0.29 mg/kg/day = 2 mg/kg/wk, 0.71 mg/kg/day = 5 mg/kg/wk, 1.43 mg/kg/day = 10 mg/kg/wk, 2.14 mg/kg/day = 15 mg/kg/wk)

Conclusions

- ✓ ELX-02 has shown pronounced restoration of CFTR activity in organoid, HBE and Ussing chamber systems
- ✓ Pharmacokinetic nonclinical and clinical results along with PB modeling data support the use of ELX-02 in CF patients with twice weekly or daily dosing regimens
- ✓ Preliminary healthy volunteer MAD pharmacokinetics are consistent with single dose (SAD) results

Acknowledgements

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References

1. Yoshizawa S, Fourmy D, Puglisi JD. Structural origins of gentamicin antibiotic action. *EMBO J* 17:6437–6448, 1998
2. Subbavarapu et al. Design of Novel Aminoglycoside Derivatives with Enhanced Suppression of Diseases-Causing Nonsense Mutations. *ACS Med. Chem. Lett.* 7(4): 418-423 (2016)
3. Shalev & T. Baasov. When proteins start to make sense: fine-tuning of aminoglycosides for PTC suppression therapy. *Med. Chem. Commun.* 5(8): 1092-1105 (2014).
4. Leubitz A, et. al. Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses of ELX-02, a Potential Treatment for Genetic Disorders Caused by Nonsense Mutations, in Healthy Volunteers. Clin Pharmacol Drug Dev. 2019 Jan 16. doi: 10.1002/cpdd.647