Translational Read-Through of CFTR Nonsense Mutations by ELX-02, A New Translational Read-Through-Inducing Drug [TRID]

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>1,800 genetic diseases involve nonsense mutations

- Single point mutation in a sequence of DNA that results in a premature stop codon resulting in a truncated, nonfunctional protein
- Nonsense mutations account for some of the most severe phenotypes in genetic diseases
- Nonsense mutations account for 12% of all genetic diseases
ELX-02: Optimized Translational Read-Through Inducing Drug (TRID)

- Fifth-generation designer aminoglycoside
- Functions as a translational read-through inducing drug (TRID)
- TRID activity optimized over the course of a decade; well-documented mechanism of action
- Promising therapeutic index in preclinical studies; successfully completed phase 1a study in healthy volunteers
- FDA and EMA orphan drug designations to treat MPS I and Rett syndrome
ELX-02

- 6'-(R)-Methyl-5-O-(5-amino-5,6-dideoxy-α-L-talofuranosyl)-paromamine sulfate
- Family of novel small molecules that increase ribosomal read-through of premature termination codons
- Improved selectivity and safety
  - Clinically relevant efficacy, satisfactory safety and no antibacterial activity
TRIDs: Full-Length, Functional Proteins

Defined Mechanism of Action

- ELX-02 stabilizes critical residues in ribosomal A-site$^{1,2}$

- Short plasma half-life, long tissue half-life, longer pharmacodynamic half-life – suitable for once weekly or twice weekly dosing

- Linear dose-response – simple dosing

- Bioavailability in CNS and other tissues


$^2$Shalev et al. Structural basis for selective targeting of leishmanial ribosomes: aminoglycoside derivatives as promising therapeutics. Nucleic Acids Research, 2015; 43(17): 8601–8613
ELX-02 Shows Efficacy in Relevant Disease Models

- ELX-02 is a potent translational read-through drug in several models of genetic disease caused by nonsense mutations.
- Repeated subcutaneous (SC) dosing of ELX-02 for 2 to 4 weeks in nonsense mutant disease animal models show:
  - Improved sensorimotor coordination of the Rett Syndrome animal model, methyl-CpG-binding protein 2 (Mecp2) with R168X nonsense mutation, $Mecp2^{R168X/x}$,
  - Increased α-L-iduronidase (IDUA) activity and decreased glycosaminoglycans accumulation in mucopolysaccharidose type I (Hurler syndrome) animal model, $Idua^{tm1Kmke}$,
  - Improved motor function and muscle force in Duchenne muscular dystrophy animal model, $mdx$,
  - Increased Cystic fibrosis transmembrane conductance regulator (CFTR) current measurements in cystic fibrosis animal model, $CFTR^{G542X/y}$.
| Cystic Fibrosis is the most prevalent genetic disease in Caucasians |
| About 10% of patients contain non-sense mutations |
| Affects Israeli patients disproportionately (~50% in Israel compared to 10% worldwide) |
| Significant unmet need, no drugs approved or in late-stage development |
| No therapeutic intervention beyond symptomatic and palliative care |
Mutations in the CFTR Transporter Channel Cause CF

Healthy cells

Cystic Fibrosis

Nonsense mutations examples:
  - G542X
  - W1282X, R553X

1. Nonsense mutations disrupt coding of the CFTR protein
2. Flow of Chloride disrupted
3. In/out flow of water, Sodium is disrupted
4. Secondary problems created by mucus buildup

Flow of Chloride disrupted

Inside cell

Outside cell

Faulty CFTR protein
ELX-02 Rescues CFTR to Clinically Relevant Levels in G542X/delF508 Human Bronchial Epithelial (HBE) Cells

* Historical data in HBE cells from healthy volunteers showed a mean value of short-circuit current 26 µA/cm² (high value of 40 µA/cm²); HBE cells were incubated for 2 days at escalating doses of ELX-02
A Combination of ELX-02 and Ivacaftor Rescues CFTR Function to Almost Normal Values in HBE Cells

- ELX-02 in combination with VX-770 (Kalydeco [Ivacaftor]) Isc levels demonstrated are about 70% of mean WT levels following 2 days of incubation
ELX-02-Dependent CFTR Activity Increases as Function of Dose and Time

Fold increase in $I_{sc}$ as function of ELX-02 dose escalation after 2 days of incubation with or without VX-770 (10µM)

Fold increase in $I_{sc}$ as function exposure time at 125µg/mL of ELX-02 with or without VX-770 (10µM)
Tobramycin Does Not Affect ELX-02 CFTR Activity in CFTR G542X/delF508 Human Bronchial Epithelial Cells

PTC 124 levels was used according to Welch et al Nature 2007; 447: 87-91

Note: Untreated primary G542X/delF508 HBE cells exhibit a residual forskolin-dependent CFTR activity of 1.1-2.9 mA/cm. These levels depend on passage number of cells and date of study.
ELX-02 Induced CFTR Rescue in FRT* CFTR Homozygous G542X and R1162X Cells

*FRT denotes for Fischer rat thyroid.
ELX-02 Increases CFTR Activity in CF Mice Model with G542X Nonsense Mutation

**p<0.01
ELX-02 Does Not Affect Antimicrobial Activity of Aminoglycosides

- ELX-02’s *in vitro* antibacterial activity was tested alone and in combination with gentamicin, tobramycin, and amikacin in the following strains:
  - *Staphylococcus aureus* (ATCC 29213),
  - *Pseudomonas aeruginosa* (ATCC 27853),
  - *Stenotrophomonas maltophilia* (ATCC 13637),
  - *Klebsiella pneumoniae* (ATCC 700603), and
  - *Escherichia coli* (ATCC 25922)

- In all strains, the average of fractional inhibitory index (FICI) of ELX-02 in combination with amikacin, gentamicin, and tobramycin ranged from 0.8 to 1.7, 0.9 to 1.4, and 1.1 to 1.7, respectively

- Results indicate that ELX-02 does not have antimicrobial activity and does not interfere with other AG’s antimicrobial activity

Note: The fractional inhibitory concentration (FIC) values were calculated for each combination then each FIC index (FICI) was calculated to assess synergistic, indifferent or antagonistic interactions.
Summary and Conclusions

- **HBE model**
  - ELX-02 demonstrated efficacy in F508del/G542X HBE cells
  - ELX-02 exhibited traditional dose response
  - Comparison to WT CFTR (26 μA/cm²), suggests that the degree of efficacy of ELX-02 is ~30% of wild type, suggesting clinical relevant efficacy
  - The addition of VX 770 to ELX-02 almost triples the efficacy in this HBE model
  - ELX-02 activity is not affected by tobramycin

- **CF G542X animal model**
  - CFTR Current measurement was significantly higher in ELX-02 treated mice (2.3±0.4) compared to PBS controls (0.5±0.04), reflecting increased CFTR activity
  - ELX-02 showed CFTR activity with potential clinical relevance

Note: WT mice Isc is (18.2±0.5)
Cystic Fibrosis Phase 2 Study

- Phase 2, open label, first-in-patient, proof of concept, multiple-dose, multi-center dose-finding study in male and female patients with cystic fibrosis caused by nonsense mutations in the CFTR gene.

- Study will evaluate the safety, tolerability, PK, PD and preliminary measures of multiple ascending SC doses of ELX-02 administered as monotherapy and in combination with ivacaftor, in independent cohorts.
EL-004: Schematic of Study

Cohort 1
n=4
0.3 mg/kg

Cohort 2
n=4
1.0 mg/kg

Cohort 3
n=4
1.0 mg/kg + Ivacaftor

Cohort 4
n=4
2.5 mg/kg

Cohort 5
n=4
2.5 mg/kg + Ivacaftor
Thank you!

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