Screening of ELX-02 Read-through Effect by FIS assay in CFTR Nonsense Mutation-bearing Organoids as Predictive Test for Clinical Trial Patient Stratification

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Poster #660

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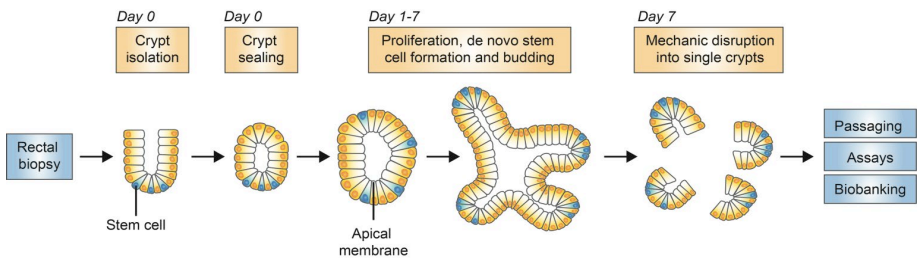
Introduction

Application of translational tools, including evaluation of patient-derived organoids*, is necessary for therapeutic development to meet the unmet need in the CF patient population, particularly those with nonsense mutations. Read-through compounds, such as the clinical stage small molecule ELX-02, are shown to induce premature stop codon read-through to produce full-length proteins. Read-through capacity is dependent on multiple factors including premature stop codon type, local *cis* regulatory factors and the codon sequence context. HIT-CF (www.hitcf.org), a collaborative project, aims to advance the access to personalized medicine for individuals with rare CF genotypes using patient-derived organoids as a translational platform to evaluate rescue of CFTR function.

HIT-CF approach

- 502 rectal biopsies were collected from European and Israeli patients with CF bearing ultra-rare mutations:
- From biopsies intestinal organoids are cultured:



- Forskolin-induced swelling (FIS) assays are performed to detect organoid response to phase I/II drugs
- 221 patient derived organoids with at least one nonsense mutation are screened with ELX-02 in three academic laboratories. 75% of the organoids carry a nonsense mutation other than G542X
- Patients for cross-over clinical trials will be selected based on organoid response

Mechanism of action of ELX-02

Figure 1 Working mechanism of ELX-02

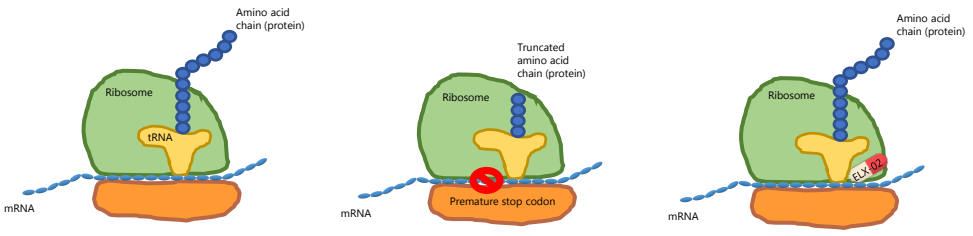
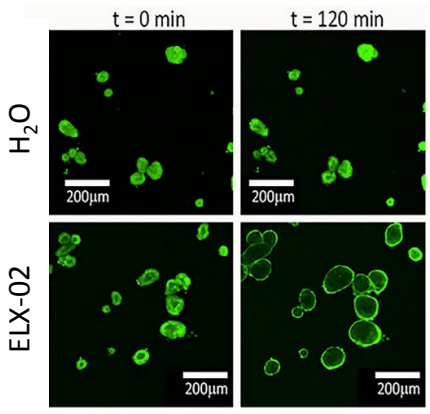
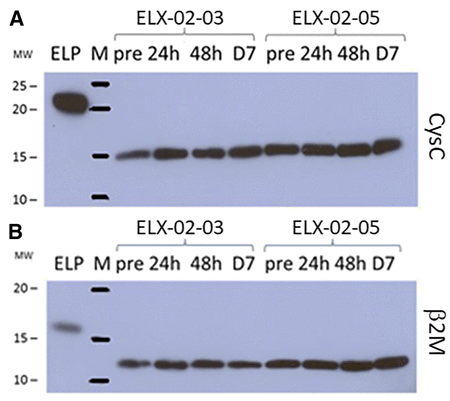


Figure 2 Example of organoid swelling upon treatment with ELX-02 on orgnanoids with G542X/G542X genotype, adapted from Crawford *et al*, 2021



Following induction with forskolin, organoids were treated with either H_2O or ELX-02 and imaged at t=0 and t=120; representative images demonstrating change in size

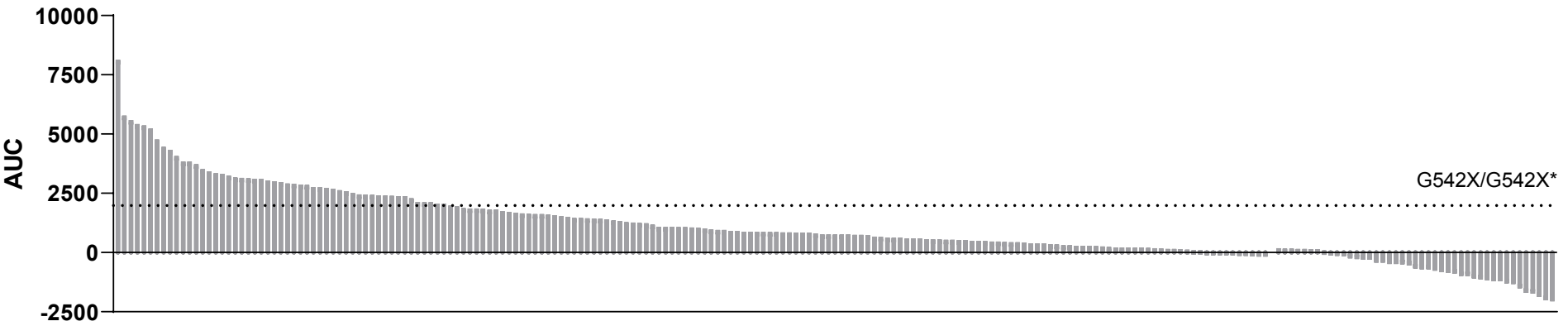
Figure 3 No evidence of native stop codon read-through in *ex vivo* clinical samples from phase I trial, adapted from Crawford *et al*, 2020



Representative western blot staining of CysC and β2M for samples from two subjects administered ELX-02 at 5.0 mg/kg. No read-through product is observed for either CysC or β2M at either predose baseline or in response to ELX-02

Results

Figure 4 Organoid response from patients with ultra-rare nonsense CFTR mutations to 80µM ELX-02, corrected for residual function (n=221)



Waterfall plot of FIS responses (0.8µM forskolin) upon 48h treatment with 80µM ELX-02. All AUC values are corrected for response on H_2O

*Average swelling response of G542X/G542X organoids

Conclusion

- A substantial proportion of patients with **ultra-rare nonsense CFTR mutations** show in vitro response to the read-through compound **ELX-02,** 25% higher than G542X/G542X genotype
- Besides G542X other (ultra)rare mutations show strong *in vitro* response

Future plans

- Highest responders will be selected for phase IIb/III trial, expected Q4 2022
- Biobank storage of all cultured organoids

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