QR-010 Restores CFTR Function in Models of ΔF508-CFTR mediated Cystic Fibrosis

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Introduction

- Cystic Fibrosis (CF) is caused by mutations in the cystic fibrosis conductance regulator (CFTR).
- The most common gene mutation is ΔF508-CFTR, which is a deletion of three nucleotides and results in a non-functional CFTR protein.
- Non-functional CFTR leads to disrupted chloride transport in multiple organs, most notably the respiratory tract and CF is characterized by thick and viscous mucus in the lungs.
- ΔF508 is a 2-C allele, fully phosphorylated T residue antisense oligonucleotide, complementary to WT CFTR mRNA aimed to restore functional CFTR in CF patients with the ΔF508-CFTR mutation.
- ΔF508-CFTR is delivered to the lungs via oral inhalation. The drug product has a neutral pH and is isosmolar.
- ΔF508-CFTR is delivered as inhaled therapy, and there are several potential biological barriers to prevent uptake by airway epithelium and systemic absorption via inhalation.
- It is hypothesized that in order for ΔF508-CFTR to be delivered via inhalation, the molecule should be stable and mobile in CF sputum.

Objectives

- Test the effect of QR-010 on ΔF508-CFTR function.
- In vivo: Nasal potential difference (NPD) measurements in ΔF508-CFTR mice.
- Assess absorption and bio distribution of QR-010 after oral-tracheal (OT) administration in wild-type (WT) mice.
- Assess in vitro and in vivo diffusion of QR-010 through CF-like mucus following repeated dosing.
- Assess if QR-010 is stable in the presence of commonly used inhaled CF Standard-of-Care Therapies.
- ΔF508-CFTR activity in: primary ΔF508-CFTR HBE cells as assessed by NPD.
- ΔF508-CFTR mice as assessed by NPD.

Materials & Methods

Short-circuit current (Isc) measurements

Homogenous ΔF508-CFTR HBE cells were grown on an air-liquid interface (ALI) and cultured in medium containing either QR-010 or a scrambled control oligonucleotide (100μM) for 3 weeks.

In vivo: The Ussing chamber assay was performed to monitor the change in short-circuit current (Isc) in response to CFTR activation and inhibition.

NPD measurements:
- Mouse ΔF508-CFTR females (FVB-Cftr tm1Eur)1 male/female, 12 weeks and older.
- Readout: NPD before treatment and after dosing at the last (6th) dose.
- Protocol: Leal et al.

In vivo and ex vivo detection of CW800-labeled QR-010

Nude mice received an oro-tracheal (OT) dose of unlabeled QR-010 or a 1:9 ratio of labeled QR-010. Mice were sacrificed at 15 minutes, 1 hour, and 3 hours. CW800-labeled QR-010 was shown in lung, liver and kidney of WT and BetaENaC mice. Repeated dosing improved signal.

In vitro diffusion of QR-010 through normal and CF-like mucus

ΔF508-CFTR activity in: primary ΔF508-CFTR HBE cells as assessed by NPD.

QR-010 diffusion speed is unchanged by repeated nebulization.

Results

- QR-010 Improves CFTR Activity in Primary ΔF508-CFTR HBE Cells
- QR-010 Restores CFTR Function in ΔF508-CFTR Mice as Assessed by NPD
- QR-010 is Stable in the Presence of Clinically Relevant Levels of CF Standard-of-Care Therapies
- Pulmonary Delivery of QR-010 Results in Systemic Exposure
- In vivo Biodistribution is Similar in WT and betaENaC Over-expressing Mice with a CF Lung Phenotype

Conclusion

- QR-010 results in functional restoration of ΔF508-CFTR activity in: primary ΔF508-CFTR HBE cells as assessed by measuring transepithelial nasal potential difference.
- ΔF508-CFTR mice as assessed by NPD.
- Pulmonary administration of QR-010 shows body-wide distribution and uptake in extra-pulmonary organs.
- QR-010 diffuses through CF-like mucus and is stable in the presence of CF Standard-of-Care Therapies.

References


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