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 non-functional CFTR protein.
- Non-functional CFTR leads to dysregulated chloride transport in multiple organ systems, most notably the respiratory tract and CF is characterized by thick and viscous mucus in the lungs.
- QR-010 is a 2'-O-methyl, fully phosphorothioated 33mer antisense oligonucleotide, complementary to WT CFTR mRNA aimed to restore functional CFTR in CF patients with the ΔF508-CFTR mutation.
- QR-010 is delivered to the lungs via oral inhalation. The drug product has a neutral pH and is iso-osmolar.
- Little is known about distribution and uptake of antisense oligonucleotides when delivered as inhaled therapy, and there are several potential biological barriers to prevent uptake by airway epithelium and/or systemic absorption via inhalation.
- It is hypothesized that in order for QR-010 to be delivered via inhalation, the molecule should be stable and mobile in CF sputum.

Objectives

- **Test the effect of QR-010 on Δ508-CFTR function:**
  - ***In vitro*: Ussing chamber short-circuit current measurements using primaryhuman bronchial epithelial cells (HBEs).**
  - ***In vivo:* Nasal epithelial potential difference (NPD) measurements in ΔF508-CFTR mice.**
- **Assess absorption and bio distribution of QR-010 after oro-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 therapies for patients with CF.**

Materials & Methods

Short-circuit current (I_{sc}) measurements
- Homozygous ΔF508-CFTR HBEs were grown on air-liquid-interface (ALI) and cultured in medium containing either QR-010 or a scrambled control oligonucleotide (100nM) for 2 weeks.
- The Ussing chamber assay was performed to monitor the change in short-circuit current (ΔIsc) in response to CFTR activation and inhibition.

NPD measurements:
- Mice: ΔF508-CFTR mice (FVB- Cftr^{tm1Eur})[1] male/female, 12 weeks and older.
- Treatment: 6x intranasal (i.n.) QR-010 (2mg/kg) every other day.
- Readout: NPD before treatment and 48hrs after the last (6th) dose.
- Protocol: Leal T et al[2].

***In vivo* and ex vivo detection of CW800-labeled QR-010**
- Nude mice received an oro-tracheal (OT), as proxy of inhalation, dose of CW800-labeled QR-010 (mixed with unlabeled QR-010 in a 1:9 ratio; total dose 10mg/kg).
- *In vivo* distribution was followed over 7 days using a Pearl *in vivo* imager (LI-COR).
- CW800-labeled QR-010 in isolated organs was detected on an Odyssey (LI-COR).

***In vitro* diffusion of QR-010 through normal and CF-like mucus**
- Clinically relevant doses of Cy5-labeled QR-010 (10, 25 or 100μM; n=2-7) were nebulized onto HBE cells, containing CF-like mucus layers (~5-11% solids)[3].
- Movement of the Cy5-labeled QR-010 through the mucus layer was assessed using a confocal microscope.

***In vivo* uptake of QR-010 in mice with CF-like lung phenotype**
- BetaENaC (Scnn1b-Tg) mice and WT littermates (n=6, 6 weeks of age) were dosed OT with 10mg/kg QR-010, 3 times per week for 2 weeks; 6 doses in total.
- At 1hr (n=3) or 24hrs (n=3) after last dosing mice were sacrificed, lung, liver and kidney were isolated and QR-010 levels were measured with a hybridization HPLC.

Compatibility of QR-010 with dornase alfa (Pulmozyme®), fluticasone, salbutamol, N-acetylcysteine and aztreonam (Cayston®)
- In test-tube mixing studies were conducted with clinically relevant concentrations in lung mucus to which QR-010 was spiked (final concentration of 10, 50 or 100μg/mL).
- Mucus was frozen immediate after spiking (t=0), and after 1hr or 24hrs at 37°C (n=2-4) and QR-010 concentration was assessed with IPRP-HPLC.

Results

QR-010 Improves CFTR Activity in Primary ΔF508-CFTR HBE Cells

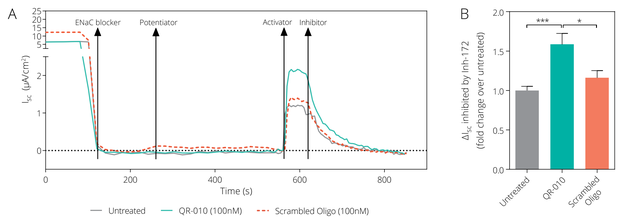

Figure 1A. I_{sc} traces measured in the Ussing chamber on filters of differentiated HBE cultures which were either untreated, or treated with QR-010 or a control oligo. 1B. CFTR activity improved significantly after treatment with QR-010 compared to non-treated cultures (***p<0.001) and to cultures treated with a negative control oligo (*p<0.05). Treatment with the negative control oligo did not result in a significantly different I_{sc} compared to non-treated cells. Mean±SEM is shown. Results were compared by one-way analysis of variance.

QR-010 Restores CFTR-Function in ΔF508-CFTR Mice as Assessed by NPD

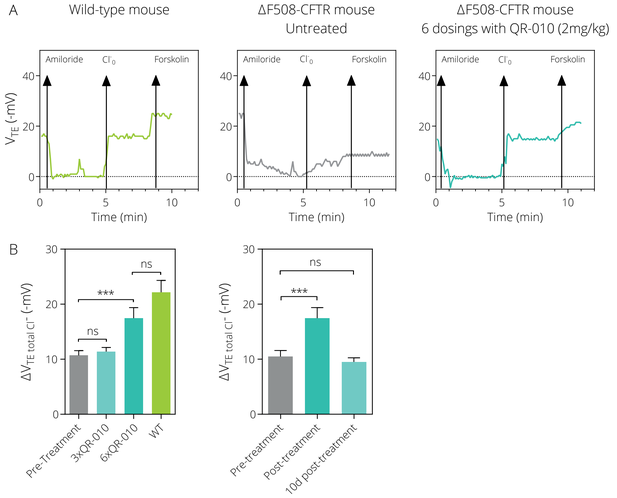

Figure 2A. Representative NPD traces of a WT mouse and a ΔF508-CFTR mouse before treatment and after 6 doses of QR-010 are shown. Both amplitude of the response and response-time to stimuli revert toward WT after QR-010 treatment. 2B. Summary of total chloride-mediated trans epithelial voltage change ($\Delta V_{TE\ total\ Cl^-}$). 3 doses of QR-010 did not significantly improve chloride response. In contrast, 6 doses of QR-010 did improve chloride response to levels not significantly different from WT. Washout effect on $\Delta V_{TE\ total\ Cl^-}$ 10 days post-treatment. Mean±SEM is shown for ΔF508-CFTR mice pre-treatment, after 3 (n=6) and 6 (n=18) i.n. doses of QR-010 (40μg/dose) and WT mice (n=6). Results were compared by unpaired T-test (vs. WT) and paired T-test (vs. pre-treatment), n=18. ***p=0.0005, ns=not significant.

QR-010 diffusion speed is unchanged by repeated nebulization

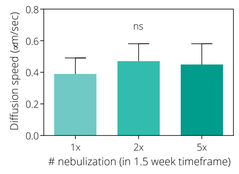

Figure 4. Diffusion speed in CF-like mucus after repeated nebulisation. No significant difference in diffusion speed of QR-010 was measured after 1, 2 or 5 times nebulization. Mean±SD is shown. ns=not significant

Pulmonary Delivery of QR-010 Results in Systemic Exposure

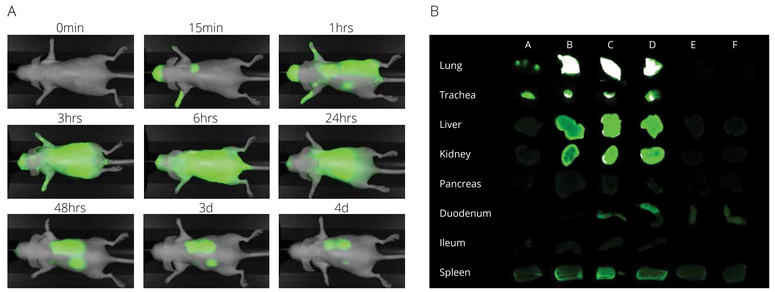

Figure 3A. In Life scans on a Pearl Impulse image system to follow distribution of CW800-labeled QR-010 over time in nude mice that received a single OT dose. 3B. CW800-labeled QR-010 detection on a Oddessey (Licor system) in several organs collected from mouse A to D at 7 days after a single OT administration. Mouse E received unlabeled QR-010 and mouse F received CW800 dye only. A strong signal of CW800-QR-010 was shown in lung, trachea, liver, kidney and spleen of mouse A to D. There was no or low signal in the control mouse E and F.

In vivo Biodistribution is Similar in WT and betaENaC Over-expressing Mice with a CF Lung Phenotype

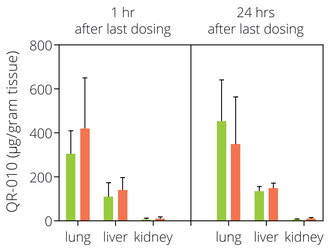

Figure 5. QR-010 levels in lung, liver and kidneys of WT and betaENaC mice. Due to overexpression of the epithelial sodium channel (ENaC), BetaENaC mice have a CF lung phenotype characterized by increased mucus concentration, mucus plugging and chronic neutrophilia. QR-010 is present in similar levels in the lungs, kidney and liver of WT and BetaENaC mice at 1hr and 24hrs after last dosing of OT administration. Mean±SD is shown.

QR-010 is Stable in the Presence of Clinically Relevant Levels of CF Standard-of-Care Therapies

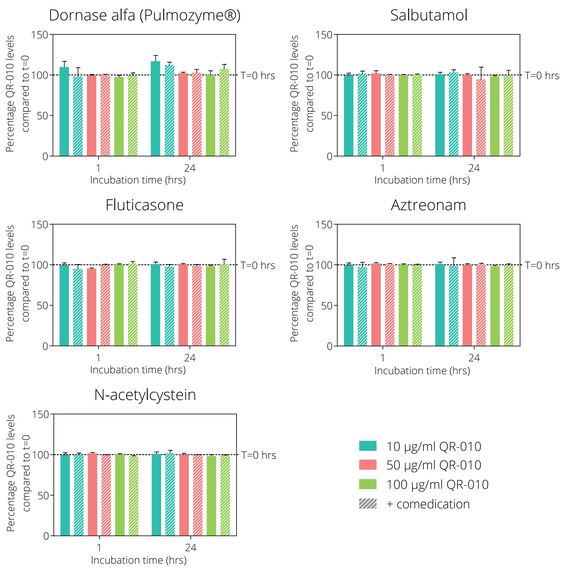

Figure 6. Normalized QR-010 levels compared to t=0 of QR-010 samples incubated with and without dornase alfa (Pulmozyme®), salbutamol, fluticasone, N-acetylcysteine or aztreonam (Cayston®). Mean±SD is shown. QR-010 levels are unaffected for at least 24hrs in the presence of clinically relevant concentrations of all five drugs.

Conclusion

- **QR-010 results in functional restoration of ΔF508-CFTR activity in:**
  - **primary ΔF508-CFTR HBE cells as assessed by Ussing chamber.**
  - **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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Thank You

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