QR-010 Penetrates the Mucus Barrier *in Vitro* and *in Vivo*

Vera Brinks[1], Katarzyna Lipinska[1], Miranda Koppelaar[1], Bianca Matthee[1], Brian Button[2], Alessandra Livraghi[3], Noreen Henig[1]

[1] ProQR Therapeutics, Darwinweg 24, 2333 CR Leiden, the Netherlands, email: vbrinks@proqr.com | [2] UNC Chapel Hill, 104 Airport Drive, Suite 2200, North Carolina, USA

[3] Marsico Lung Institute, University of North Carolina at Chapel Hill, 125 Mason Farm Rd, Chapel Hill, NC 27599, USA

Introduction

- Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis conductance regulator (CFTR). The most common gene mutation is ΔF508, which results in the deletion of three nucleotides and in non-functional CFTR protein.
- Non-functional CFTR results in dysregulated chloride transport in multiple organ systems, most notably the respiratory tract.
- CF is characterized by thick and viscous mucus in the lungs. Patients with CF and pulmonary exacerbations have polymicrobial infections, active inflammation, and sputum that contains increased amounts of proteases, DNases, cell debris, multiple inhaled therapeutic molecules, and mucins.[1]
- The CF lung mucus/sputum layer is considered a potential barrier in lung delivery because of the dense network of glycosylated proteins-mucins and microbial and inflammatory characteristics.
- Dornase alfa (Pulmozyme®), fluticasone, and salbutamol are inhaled therapies commonly used to treat symptoms of CF.
- QR-010 is a 2' O methyl, fully phosphorothioated 33mer antisense oligonucleotide, complementary to wild type CFTR mRNA aimed to repair mRNA in CF patients with the ΔF508 mutation, and to restore functional CFTR.
- QR-010 is delivered to the lungs via oral inhalation. The drug product is neutral pH and isoosmolar.
- 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

- **Assess if QR-010 is stable in sputum collected from patients with CF.**
- **Assess in vitro diffusion of Cy-5 labeled QR-010 through normal and CF-like mucus following nebulization on primary human lung epithelial cell cultures.**
- **Assess in vivo diffusion of QR-010 through CF-like lung mucus in the betaENaC mice (Scnn1b-Tg), a mouse model with a CF-lung phenotype.**
- **Assess if QR-010 is stable in the presence of commonly used inhaled therapies for patients with CF: dornase alfa (Pulmozyme®), fluticasone, and salbutamol.**

Material and Methods

Stability in CF sputum

- Human CF sputum pools were obtained from stable patients during routine visits.
- QR-010 was spiked to a final concentration of either 10 or 100 µg QR-010/ml sputum.
- Incubation times were: control, immediate freezing after spiking (t=0), 12 hrs, 24 hrs or 48 hrs at 37 °C (n=7).
- QR-010 concentrations were measured with a dual hybridization ELISA.

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

- Air liquid interface (ALI) cultures of primary human bronchial epithelial (HBE) cells, containing normal (~2-5% solids) or CF-like mucus layers (~5-11% solids) were used.[2]
- A volume of 100 nl Cy-5 labeled QR-010 was nebulized onto the mucus layer of ALI cultures. The height of the mucus layers ranged between ~15-30 µm.
- Different doses of clinically relevant Cy-5 QR-010 were nebulized: 10µM, 25µM or 100µM (n=2-7).
- Movement of the Cy-5 labeled QR-010 through the mucus layer and the time to reach a fluorescence intensity of 60% at the epithelial cell were assessed using a XZ laser scanning confocal microscope.

In vivo uptake of QR-010 in mice with CF-like lung phenotype

- In contrast to the CFTR mutant mice, betaENaC mice (Scnn1b-Tg) have a CF lung phenotype characterized by increased mucus concentration, mucus plugging and chronic neutrophilia.
- BetaENaC mice and wildtype littermates (WT) (n=6, 6 weeks of age) were dosed orotracheally with 10 mg/kg QR-010. Neither the WT or betaENaC mice had acute or chronic airway infection.
- Mice were dosed 3 times per week for 2 weeks; 6 doses in total.
- At 1 hr (n=3) or 24 hrs (n=3) after last dosing mice were sacrificed and lung, liver and kidney were isolated.
- QR-010 levels were measured with a hybridization HPLC.

Compatibility of QR-010 with dornase alfa (Pulmozyme®), fluticasone and salbutamol

- In test-tube mixing studies were conducted with clinically relevant concentrations in the lung mucus:
  - Dornase alfa (Pulmozyme®): 3 µg/ml
  - Fluticasone: 5 µg/ml
  - Salbutamol: 2 µg/ml
- QR-010 was spiked to a final concentration of 10, 50 or 100 µg/ml, reflecting expected clinically relevant concentrations in the lung mucus.
- Incubation times were: control, immediate freezing after spiking (t=0), 1 hr or after 24 hrs at 37 °C (n=2-4), reflecting the maximal duration QR-010 might be in contact with one of these drugs.
- QR-010 concentration and presence of breakdown products was assessed with IPRP-HPLC.

Results

QR-010 levels remain stable in human CF sputum for at least 48 hrs

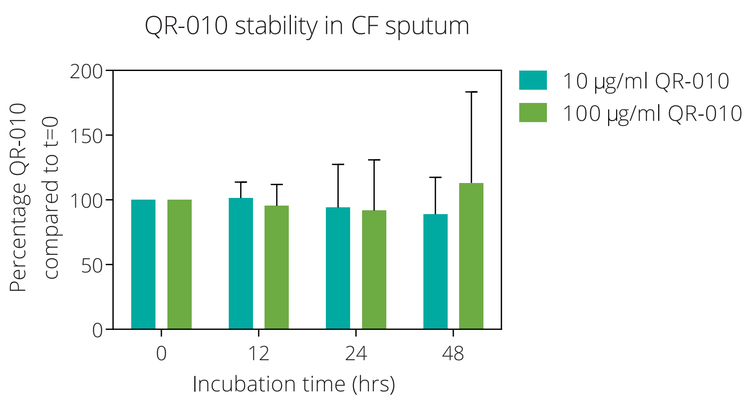

Figure 1. Stability of QR-010 in sputum from CF patients. Error bars show standard deviation. QR-010 concentration is unaffected for at least 48 hrs in CF sputum.

In vitro diffusion of QR-010 through normal (low solute) and CF-like (high-solute) mucus is rapid and not significantly impacted by solute concentration

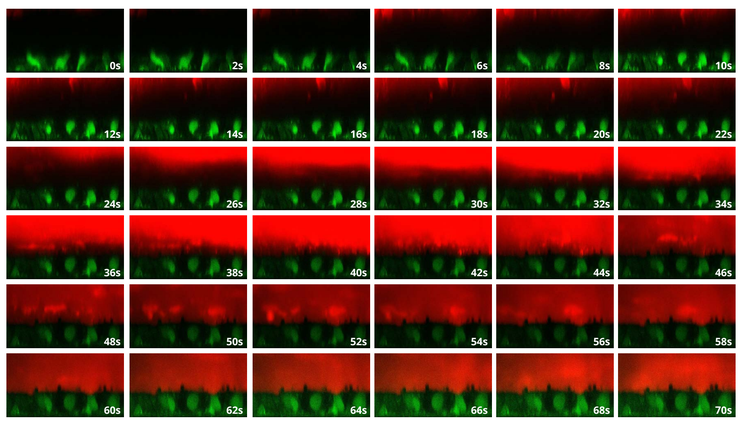

Figure 2. Time-lapse: Representative XZ images of Cy-5 QR-010 (red; 100 µM) diffusion through a normal in vitro mucus layer. Green indicates calcein stained cells. Images go from upper left to lower right, time between pictures is 2 seconds each. Diffusion of Cy-5 QR-010 is not blocked by the mucin network.

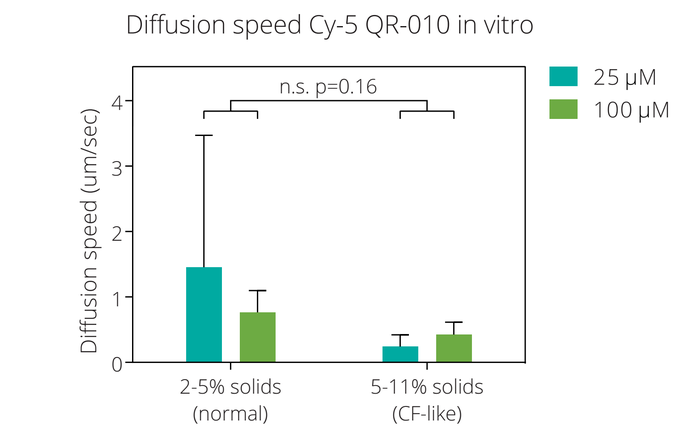

Figure 3a and b. Diffusion speed (a) and time to reach 60% Cy-5 QR-010 signal at the epithelial cells (b). Error bars show standard deviation. Movement of the Cy-5 labeled QR-010 was not possible to track for the lowest (10 µM) dose level. Diffusion speed of Cy-5 QR-010 ranges between ~0.2-3 µm/sec, and within minutes after nebulization 60% Cy-5 QR-010 levels at the epithelial cell layer is reached. The diffusion speed and time to reach 60% Cy-5 QR-010 signal at the epithelial cells layer is similar between normal and CF-like mucus and is unaffected by Cy-5 QR-010 dose.

In vivo uptake of QR-010 is similar between WT and betaENaC mice (CF lung phenotype)

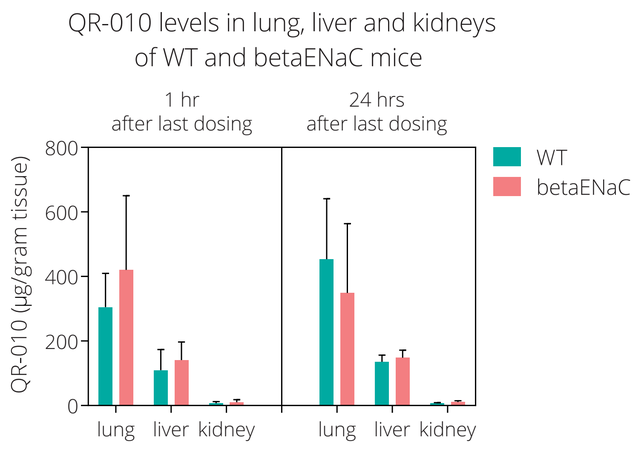

Figure 4. QR-010 levels in lung, liver and kidneys of WT and betaENaC mice. Error bars show standard deviation. QR-010 is present in similar levels in the lungs, kidney and liver of WT and Scnn1b-Tg mice at 1 hr and 24 hrs after last dosing via orotracheal application.

QR-010 is stable for at least 24hrs in the presence of clinically relevant levels of dornase alfa (Pulmozyme®), fluticasone or salbutamol

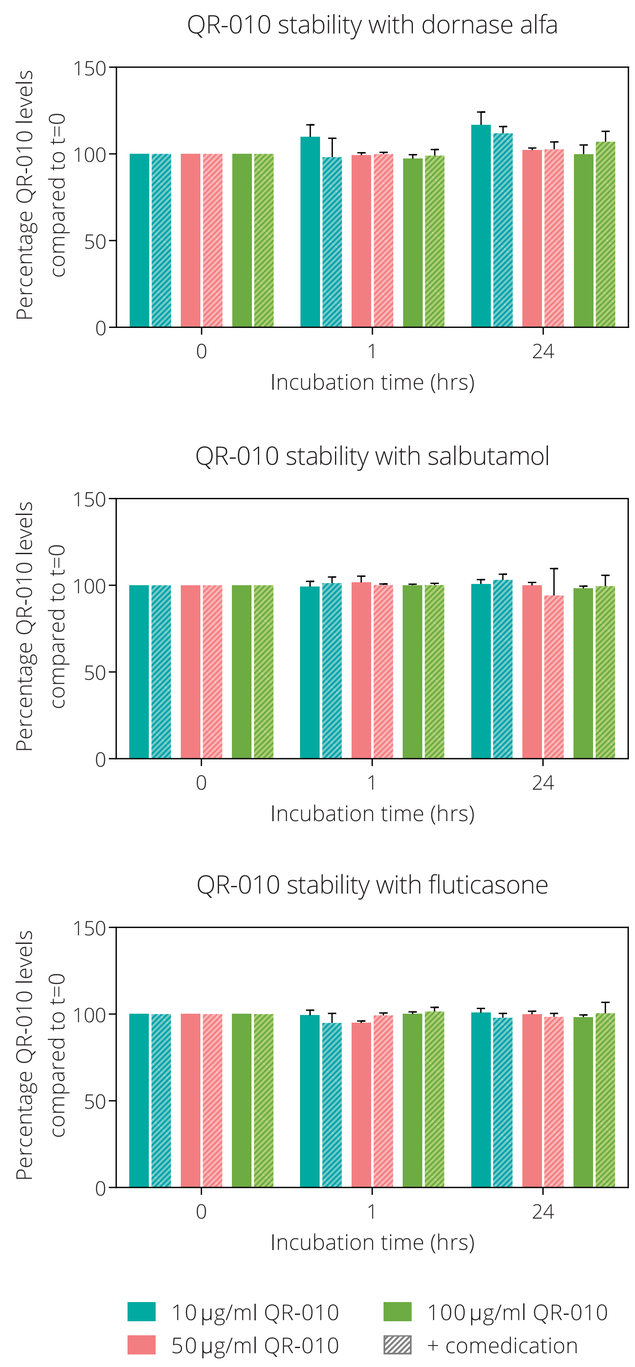

Figure 6a, b and c. Normalized QR-010 levels compared to t=0 of QR-010 samples incubated with and without dornase alfa (Pulmozyme®), salbutamol or fluticasone. Error bars show standard deviation. QR-010 levels are unaffected for at least 24 hrs in the presence of clinically relevant concentrations of all three drugs.

Conclusions

- **QR-010 is stable in sputum collected from CF patients.**
- **QR-010 easily diffuses through CF-like mucus in vitro and in vivo in a clinically relevant timeframe. Solute percentage in the mucus will slow diffusion slightly but it is not a meaningful difference.**
- **QR-010 is absorbed systemically after orotracheal administration in WT and betaENaC mice. Quantitative assays show no difference between WT and betaENaC mice consistent with the findings that CF like ALI does not inhibit QR-010 diffusion.**
- **QR-010 stability is not affected by dornase alfa (Pulmozyme®), fluticasone or salbutamol.**

Acknowledgements

We thank Axolabs GmbH for the analysis of mouse samples using the hHPLC method.

References

1. Rubin BK. 2009. Mucus, phlegm, and sputum in cystic fibrosis. Respir Care. Jun;54(6):726-32; discussion 732.
2. Hill DB, Vasquez PA, Mellnik J, McKinley SA, Vose A, Mu F, Henderson AG, Donaldson SH, Alexis NE, Boucher RC, Forest MG,. 2014. A biophysical basis for mucus solids concentration as a candidate biomarker for airways disease. PLoS One. Feb 18;9(2).

Scan the code for a digital copy