**Therapeutic Inhibition of ENaC With a Lung-Targeted RNAi Molecule Delivery Platform Preserves Normal Mucus Clearance in a Muscovet Sheep Model of Cystic Fibrosis**

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**Abstract:**
In cystic fibrosis (CF), increased epithelial sodium channel (ENaC) activity accompanies loss of CFTR channel function and contributes to airway mucus hypersecretion. Loss-of-function ENaC mutations increase mucociliary clearance (MCC) and modify CF lung disease to milder phenotypes, whereas gain-of-function alleles contribute to the pathogenesis of atypical CF. Despite strong genetic and functional validation of the target, clinical development of inherited small molecule ENaC inhibitors has been limited by their short duration of action and renal side effects. Using Arrowhead’s Targeted RNAi Molecule (TRiM)™ technology, we developed ARO-ENaC (an optimized RNA trigger against ENaC mRNA paired with a pulmonary epithelial targeting ligand), which has previously been shown to durably and selectively silence ENaC expression in the rodent lung. In the current study, we evaluated the effects of ENaC silencing on large animal lung physiology. Mucociliary clearance was measured in ARO-ENaC-treated normal sheep and in sheep challenged with neutrophil elastase, an ENaC activating protease that promotes airway liquid depletion, increased mucus secretion and mucostasis mimicking CF lung disease.

**METHODS:**
- Male Sprague Dawley rats received aerosolized ARO-ENaC conjugate or ENaC trigger alone, delivered via jet nebulizer and nose-only inhalation. One week after dosing, total RNA was isolated from whole lung homogenates and mRNA expression analyzed by qRT-PCR following TRiM™ delivery platform increases ENaC mRNA silencing following aerosol dosing in rats

**RESULTS:**
- At baseline, sheep had normal MCC. ENaC silencing approximately doubled MCC at Day 14 and 28.
- At Day 1, sheep received a single inhaled dose of ARO-ENaC. Follow-up MCC scans were performed weekly.
- A single 1.7 mg/kg dose of ARO-ENaC approximately doubled MCC at Day 28.
- At Day 21, MCC in 17.6 mg/kg group remained partially elevated (50% above baseline). Clearances remained at twice baseline for sheep receiving 2.4 mg/kg and 5.2 mg/kg ARO-ENA.

**CONCLUSIONS:**
- Neutrophin elastase causes complete mucostasis (dotted line), with no tracer cleared over two hours.
- Sheeps receiving three inhaled doses of 0.5 mg/kg ARO-ENA two weeks prior to neutrophil elastase challenge (red line) were fully protected from mucostasis, maintaining lung clearance approximately 25% above their pretreatment baselines.

**REFERENCES:**
10. Sabater et al. (2017) JCI Insight 2:208464

**TRiM™ platform**
- Rules and algorithms allow selection of optimized RNA trigger sequences.
- Limit cross-reactivity with off-target genes.
- Maximize innate stability.
- Rational-use and placement of modifying chemicals.
- Active rinse-out to minimize delivery to target tissues.
- In vivo targeting minimizes delivery to target tissues.
- In vivo targeting regulatory silencing of ENaC required.
- Targeting ligands and linker chemistries improve delivery to target tissues.
- Accelerated MCC could be observed one week after ARO-ENaC inhalation.
- Inhaled RNAs trigger without targeting ligand did not accelerate MCC.
- Similarly, three daily inhaled dosed doses of 0.5 mg/kg ARO-ENaC doubled lung MCC in normal sheep two weeks after the third dose.

**ARO-ENaC preserves airway physiology in a sheep disease model of impaired mucociliary clearance**
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- Neutrophin elastase causes complete mucostasis (dotted line), with no tracer cleared over two hours.
- Sheeps receiving three inhaled doses of 0.5 mg/kg ARO-ENA two weeks prior to neutrophil elastase challenge (red line) were fully protected from mucostasis, maintaining lung clearance approximately 25% above their pretreatment baselines.

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