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

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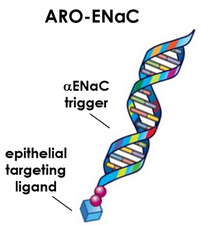
RATIONALE

In cystic fibrosis (CF), increased epithelial sodium channel (ENaC) activity accompanies loss of CFTR channel function and contributes to airway dehydration and mucoobstruction[1]. Loss-of-function ENaC alleles increase mucociliary clearance (MCC)[2] and modify CF lung disease to milder phenotypes[3], while gain-of-function alleles contribute to the pathogenesis of atypical CF[4]. Despite strong genetic and functional[5] validation of the target, clinical development of inhaled small molecule ENaC inhibitors has been limited by their short duration of action and renal side effects[6]. Using Arrowhead's Targeted RNAi Molecule (TRiM™) technology, we developed ARO-ENaC (an optimized RNAi trigger against αENaC mRNA paired with a pulmonary epithelial targeting ligand), which has previously been shown to durably and selectively silence αENaC expression in the rodent lung[7]. In the current study, we evaluated the effects of αENaC 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 surface liquid depletion, increased mucus secretion and mucostasis mimicking CF lung disease.

METHODS

- Male Sprague Dawley rats received aerosolized ARO-ENaC conjugate or αENaC 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 qPCR.
- Baseline mucociliary clearance (MCC) in conscious, nasally intubated female sheep (n=3 per group) was measured by inhalation of aerosolized technetium-labeled sulfur colloid (^{99m}Tc-SC) followed by gamma imaging at five minute intervals for two hours.
- Aerosolized ARO-ENaC conjugate or αENaC trigger alone was delivered to nasally intubated sheep (either one dose or three daily doses). Follow-up mucociliary clearance scans were performed weekly at 7, 14, 21 and 28 days post-dose.
- For the impaired MCC model, human neutrophil elastase (HNE) was administered by inhalation immediately prior to gamma imaging[8].
- Deposited pulmonary doses were calculated using the respiratory minute volume, aerosol concentration, exposure time, body weight and deposition fraction (10% for rat; 25% for sheep).

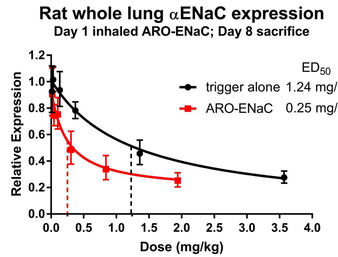
TRiM™ platform



- Rules and algorithms allow selection of optimized RNAi trigger sequences
- Limit cross-reactivity with off-target genes
- Maximize innate stability
- Rational use and placement of modifying chemistries
- Active endosomal escape chemistries not required
- Targeting ligands and linker chemistries improve delivery to target tissues
- Integrin αvβ6 targeting moiety facilitates pulmonary epithelial uptake and endocytosis of triggers

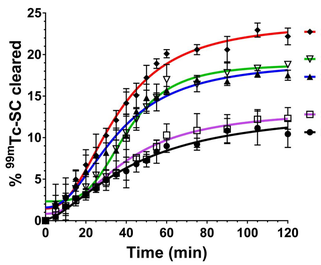
RESULTS

Epithelial targeting ligand increases potency of whole lung αENaC mRNA silencing following inhaled aerosol dosing in rats

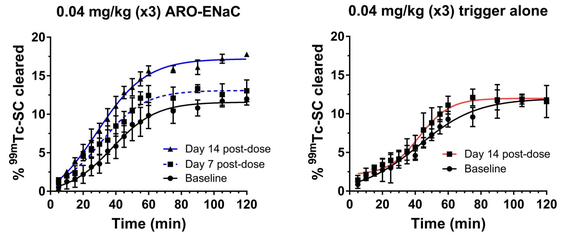


- Rats received a single inhaled aerosol dose of ARO-ENaC conjugate (RNAi trigger + ligand) or trigger alone. Whole lung αENaC mRNA expression was evaluated one week post-dose
- ARO-ENaC's epithelial targeting ligand increases potency of trigger

Dose-dependent acceleration of mucociliary clearance (MCC) in normal sheep two weeks after ARO-ENaC inhalation

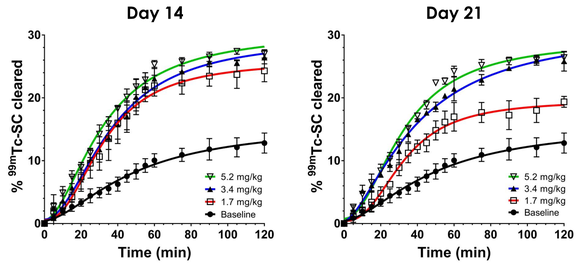


- At baseline, sheep cleared approximately 10-12% of inhaled tracer
- On Days 1-3, ARO-ENaC was inhaled at 0.5, 0.22, 0.04, and 0.02 mg/kg deposited dose levels
- On Day 17, accelerated MCC was observed at all doses: +113% above baseline (0.5 mg/kg), +80% (0.22 mg/kg), +68% (0.04 mg/kg) and +18% (0.02 mg/kg)



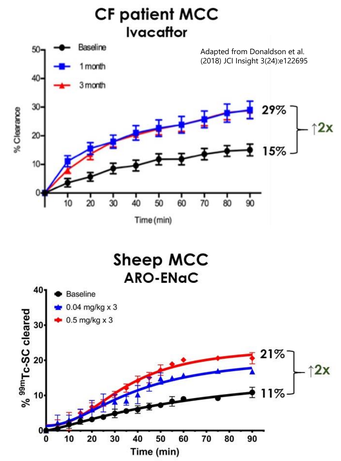
- Accelerated MCC could be observed one week after ARO-ENaC inhalation
- Inhaled RNAi trigger without targeting ligand did not accelerate MCC

A single inhaled dose of ARO-ENaC accelerates mucociliary clearance in normal sheep up to three weeks



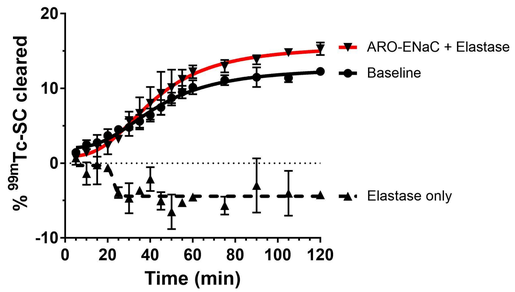
- On Day 1, sheep received a single inhaled dose of ARO-ENaC. Follow-up MCC scans were performed weekly
- A single 1.7 mg/kg dose of ARO-ENaC approximately doubled MCC at Day 14
- At Day 21, MCC in 1.7 mg/kg group remained partially elevated (50% above baseline). Clearances remained at twice baseline for sheep receiving 3.4 mg/kg and 5.2 mg/kg ARO-ENaC
- By Day 28, MCCs returned to baseline (or near baseline) for all exposure levels

αENaC silencing improves mucociliary clearance with a benefit similar in magnitude to ivacaftor in G551D-CFTR CF patients



- As previously shown by Donaldson et al.[9], lung MCC doubled in G551D-CFTR CF patients within one month of initiation of ivacaftor treatment (150 mg, BID)
- In ivacaftor-treated patients, accelerated MCC correlated with improved lung function, measured by FEV_1
- Similarly, three daily inhaled deposited 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



- Neutrophil elastase cleaves and activates near-silent pulmonary epithelial ENaC channels[10] and may also increase mucus secretion, contributing to airway surface liquid depletion, mucus hyperconcentration and impaired clearance
- When administered to sheep via inhalation, neutrophil elastase caused complete mucostasis (dotted line), with no tracer cleared over two hours
- Sheep receiving three inhaled doses of 0.5 mg/kg ARO-ENaC two weeks prior to neutrophil elastase challenge (red line) were fully protected from mucostasis, maintaining lung clearance approximately 25% above their pretreatment baselines

CONCLUSIONS

- Arrowhead's pulmonary epithelial TRiM™ delivery platform increases potency of RNAi trigger-mediated silencing of αENaC mRNA, durably reducing whole lung gene expression in rodents
- In normal sheep, inhaled ARO-ENaC produces dose-dependent acceleration of mucociliary clearance, a lung physiology endpoint linked to pulmonary function in CF patients
- Inhaled ARO-ENaC preserves lung clearance in a sheep disease model of mucostasis caused by challenge with the ENaC-activating protease neutrophil elastase
- ARO-ENaC offers a new renal-sparing, genotype-agnostic mucokinetic therapy for all CF patients, with an extended duration of action that should minimize treatment burden. IND/CTA-enabling studies are in process to support regulatory filings for first-in-human studies
- Arrowhead is expanding the pulmonary delivery platform to address additional disease targets, particularly those that are inaccessible to traditional small molecule or antibody approaches

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