A Novel Targeted RNAi Molecule Delivery Platform for the Therapeutic Inhibition of ENaC in Cystic Fibrosis Lung Disease

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RATIONAL
In cystic fibrosis (CF), mutations in the CTR chloride channel gene are associated with increased epithelial sodium channel (ENaC) activity which contributes to airway dehydration and reduced airway mucociliary transport. Hypertonically, ENaC alleles increase mucociliary transport and modify CF lung disease to milder phenotypes, but the development of inherited small molecule inhibitors has been limited by their short duration of action and side effects resulting from renal ENaC inhibition. To enable durable, renal-sparing therapeutic ENaC inhibition for CF genotype, we have utilized Arrowhead’s Targeted RNAi Molecule (TRiM™) technology to develop an epithelial-targeted conjugate comprised of an optimized RNAi trigger against the ENaC mRNA paired with an epithelial targeting ligand (EpL) to the integrin α6β4 receptor.

METHODS
- In vitro and in vivo uptake was evaluated by fluorescence microscopy. Tracking conjugates were pre-conjugated by conjugating the EpL ligand to a Cy3-labeled uRNA trigger.
- Normal human bronchial epithelial cells were cultured at the air-liquid interface for 4 weeks prior (5 x 10^5 cells/3 cm², 3曝 exposures to 5 μM conjugate). Using chambers were used to measure the amiloride-dependent short-circuit current (Isc) 1 week post-dose. Airway surface liquid volume was measured by meniscus scanning.
- Rats received oropharyngeal (OP) doses of EpL-RNAi conjugate ADS-003 or TRiM RNA trigger alone, total RNA was isolated from whole lung and kidney homogenates and mRNA expression analyzed by qPCR.
- For inhalation studies, 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). Using the respiratory minute volume, aerosol concentration, exposure time, body weight and deposition fraction (10% for rat; 25% for sheep).
- An Aerosol Six mesh nebulizer or MityMax jet nebulizer was used for rat or sheep studies, respectively.
- Mucociliary clearance in normal, conscious sheep was measured by inhalation of aerosolized technetium-labeled sulfur colloid (99mTc-SC) followed by gamma imaging at 5 minute intervals for 2 hours.

RESULTS
Epithelial targeting α6β4 ligand (EpL) facilitates uptake of an ENaC RNAi trigger by human bronchial epithelial cells in vitro
- HBE exposed to Epi-trigger conjugate (ADS-003) expressed 40% less ENaC mRNA, with reduced sodium channel activity and increased ASL volume
- EpL-RNAi trigger conjugate is internalized by rat bronchiolar epithelial cells in vivo after oropharyngeal delivery

EpL-ENaC trigger conjugate mediates durable whole lung ENaC mRNA silencing
- Rats received three q.d. inhaled aerosolized ENaC mRNA expression or serum potassium levels were observed
- ADS-003 was well tolerated with no significant findings in clinical chemistry, hematology or histopathology

TRiM™ platform
- ARO-ENAc
- EpL targeting EnaC
- Integrin with C2 domain moiety facilitates pulmonary epithelial uptake and endocytosis of triggers

Inhalation of aerosolized EpL-ENaC trigger conjugate increases airway mucociliary clearance in sheep two weeks post-dose
- After baseline MCC measurements, sheep received inhaled doses of ADS-003 (n=3 sheep per dose level) on Days 1-3. On Day 17, the 0.07 and 0.35 mg/kg group had MCC values approximately 7-8% above baseline, while the 0.7 mg/kg group remained approximately 12% above baseline MCC values
- Inhaled amiloride (3 ml 3 mM n=2 sheep) was administered immediately prior to scanning to its short (1-2 hour) duration of action. Amiloride acutely increased MCC approximately 32% above baseline MCC values

CONCLUSIONS
- An integrin α6β4 receptor ligand (EpL) improves endocytosis of RNAi triggers by human bronchial epithelial cells and by the rat airway epithelium
- The EpL-ENaC trigger conjugate ADS-003 improves functional delivery to the airway epithelium and facilitates selective, durable, renal-sparing silencing of lung ENaC mRNA expression
- ADS-003 dose-dependently increases mucociliary clearance in sheep two weeks after inhalation, with the lowest dose matching the previously reported increased clearance effect of acutely administered hypertonic saline
- The extended duration of action of ADS-003 may provide a benefit over inhaled small molecule ENaC inhibitors which transiently block channel function
- ARO-ENAc for cystic fibrosis is Arrowhead’s first pulmonary program to employ the TRiM™ platform. Additional therapeutic targets in the pulmonary epithelium could be considered, particularly those that are currently inaccessible to traditional small molecule or antibody approaches

REFERENCES
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