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

Erik W. Bush[1], Anthony Nicholas[1], Xiaokai Li[1], Ine Kuipers[1], Holly Hamilton[1], Julia Hegge[1], Rui Zhu[1], Bo Chen[1], Thomas Schluep[2], Nathalie Baumlin[3], Matthias A. Salathe[3], Ren-Jay Shei[4], Steven M. Rowe[4], Burton F. Dickey[5], Juan R. Sabater[6], Marcus A. Mall[7], Zhen Li[1]

1 Arrowhead Pharmaceuticals Inc., Madison, WI, United States; 2 Arrowhead Pharmaceuticals Inc., Pasadena, CA, United States; 3 University of Kansas, Kansas City, KS, United States; 4 University of Alabama at Birmingham, Birmingham, AL, United States; 5 Department of Pulmonary Medicine, University of Texas M. D. Anderson Cancer Center, Houston, TX, United States; 6 Mount Sinai Medical Center, Miami Beach, FL, United States; 7 Department of Pediatric Pulmonology, Immunology and Intensive Care Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany

arrowhead pharmaceuticals

NORTH AMERICAN CYSTIC FIBROSIS CONFERENCE

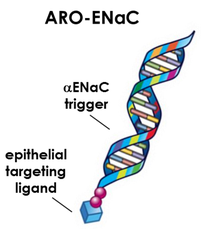
RATIONALE

In cystic fibrosis (CF), mutations in the CFTR chloride channel gene are associated with increased epithelial sodium channel (ENaC) activity which contributes to airway dehydration and reduced airway mucociliary transport[1]. Hypomorphic ENaC alleles increase mucociliary transport[2] and modify CF lung disease to milder phenotypes[3], but the development of inhaled small molecule inhibitors has been limited by their short duration of action and side effects resulting from renal ENaC inhibition[4]. To enable durable, renal-sparing therapeutic ENaC inhibition for all CF genotypes, we have utilized Arrowhead's Targeted RNAi Molecule (TRiM™) technology to develop an epithelial-targeted conjugate comprised of an optimized RNAi trigger against the αENaC mRNA paired with an epithelial targeting ligand (EpL) to the integrin αvβ6 receptor[5].

METHODS

- *In* vitro and *in vivo* uptake was evaluated by fluorescence microscopy. Tracking conjugates were prepared by conjugating the EpL ligand to a Cy3-labeled αENaC RNAi trigger.
- Normal human bronchial epithelial cells were cultured at the air-liquid interface for 4 weeks prior to conjugate exposure (5 x q.d. 3-hour exposures to 5 μM conjugate). Ussing 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 trigger conjugate ADS-003 or RNAi 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). An AeroNeb Solo mesh nebulizer or MistyMax 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 (^{99m}Tc-SC) followed by gamma imaging at 5 minute intervals for 2 hours.

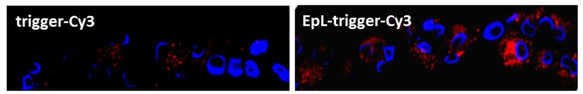
TRiM™ platform



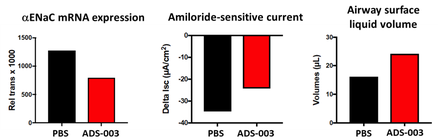
- Rules and algorithms allow selection of optimized RNAi trigger sequences
- Limit cross-reactivity with off-target genes
- Maximize innate stability
- Rational use and placement of modifying chemistries
- Active endosomal escape chemistries not required
- Targeting ligands and linker chemistries improve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

RESULTS

Epithelial targeting αvβ6 ligand (EpL) facilitates uptake of an αENaC RNAi trigger by human bronchial epithelial cells *in vitro*

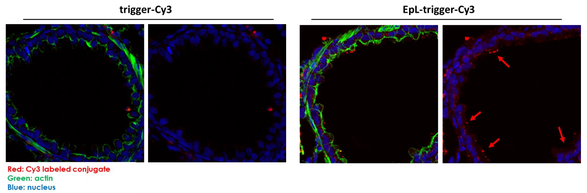


- EpL ligand improved baseline uptake uptake of Cy3-labeled αENaC trigger by human bronchial epithelial (HBE) cells in air-liquid interface culture



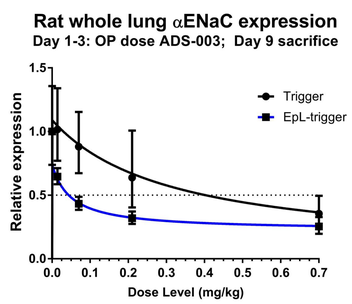
- HBE exposed to EpL-trigger conjugate (ADS-003) expressed 40% less αENaC mRNA, with reduced sodium channel activity and increased ASL volume

EpL-αENaC trigger conjugate is internalized by rat bronchiolar epithelial cells *in vivo* after oropharyngeal delivery



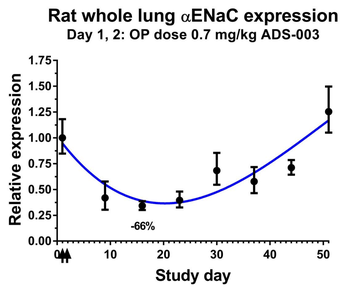
- Rats received an OP dose of 0.1 mg/kg of Cy3-labeled trigger and were sacrificed 24 hours later. Endocytosis of labeled trigger by airway epithelial cells (punctate signal, arrows) was improved by conjugation to EpL ligand

Targeting ligand increases trigger potency 10x and improves uniformity of αENaC mRNA silencing in the lung



- Rats received three q.d. OP doses of αENaC RNAi trigger alone or EpL-trigger conjugate (ADS-003) and sacrificed 6 days after last dose
- EpL targeting increased potency and uniformity of whole lung αENaC mRNA silencing, consistent with improved delivery to airway epithelium

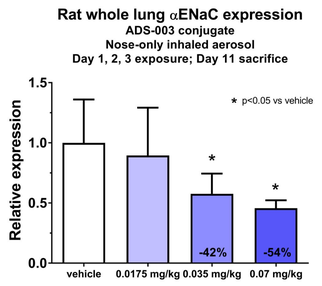
EpL-αENaC trigger conjugate mediates durable whole lung αENaC mRNA silencing

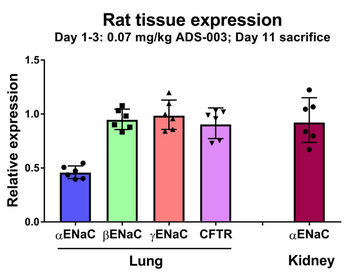


- Rats received two q.d. OP doses of EpL-trigger conjugate (ADS-003); treatment groups were sacrificed weekly
- Maximum reduction in rat lung αENaC mRNA (nadir) is 2 weeks after dosing; expression fully recovers between 6-7 weeks post-dose
- Durability of silencing supports an every other week (or less frequent) maintenance dose regimen

Inhalation of aerosolized EpL-αENaC trigger conjugate selectively silences pulmonary αENaC mRNA expression with no effect on renal expression

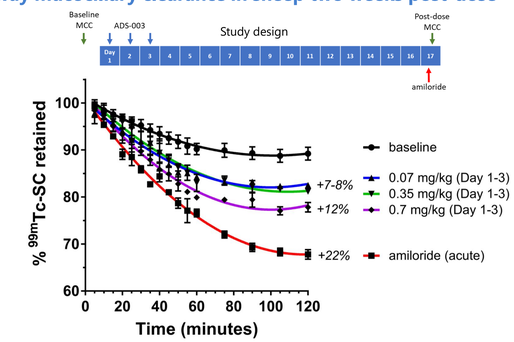
- Rats received three q.d. inhaled aerosol exposures of isotonic saline (vehicle) or ADS-003 conjugate (estimated 0.0175, 0.035 or 0.07 mg/kg deposited dose) and sacrificed 8 days after last dose
- Whole lung αENaC mRNA expression was reduced by 54% after three inhaled doses of 0.07 mg/kg ADS-003





- ADS-003 selectively silenced lung expression of the αENaC subunit mRNA. Expression of the homologous βENaC and γENaC subunits and CFTR mRNA was unchanged
- No changes in renal αENaC mRNA expression or serum potassium levels were observed
- ADS-003 was well-tolerated with no significant findings in clinical chemistry, hematology or histopathology

Inhalation of aerosolized EpL-αENaC 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 groups 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 scan due to its short (1-2 hour) duration of action. Amiloride acutely increased MCC approximately 22% above baseline MCC values

CONCLUSIONS

- An integrin αvβ6 receptor ligand (EpL) improves endocytosis of RNAi triggers by human bronchial epithelial cells and by the rat airway epithelium
- The EpL-αENaC trigger conjugate ADS-003 improves functional delivery to the airway epithelium and facilitates selective, durable, renal-sparing silencing of lung αENaC 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[7]
- 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

1. Hobbs C et al. (2013) J Physiol 591:4377-4387
2. Kerem E et al. (1999) N Engl J Med 341:156-161
3. Agrawal P et al. (2017) Am J Respir Cell Mol Biol 57:711-720
4. O'Riordan T et al. (2014) J Aerosol Med Pulm Drug Deliv 27:200-208
5. Sheppard D (2003) Physiol Rev 83:673-686
6. Enuka Y et al. (2012) Histochem Cell Biol 137:339-353
7. Coote K J et al. (2015) Br J Pharmacol 172:2814-2826

Presented at NACFC 2018