Targeting αENaC with an epithelial RNAi trigger delivery platform for the treatment of cystic fibrosis

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Disclosures

• I am an employee and shareholder of Arrowhead Pharmaceuticals, Inc.
Increased epithelial sodium channel (ENaC) activity promotes mucus dehydration in cystic fibrosis lung disease

- Hypomorphic alleles of ENaC subunits increase mucociliary transport, resulting in milder CF phenotypes
- ENaC inhibitors promise pan-genotypic approach, but small molecules have encountered challenges in clinic

"The rational design of new ENaC blockers must include not only the provision of a sustained increase in mucociliary clearance, but also the avoidance of clinically significant renal exposure..."

O'Riordan 2014
TRiM™ platform: Targeted RNAi Molecules

ARO-ENaC

• 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 ligands facilitate uptake and endocytosis of triggers by pulmonary epithelium

EpL = integrin αvβ6 ligand
Epithelial targeting ligands (EpL) facilitate RNAi trigger internalization by integrin αvβ6+ cells in vitro

Receptor internalization
On-cell Western assay

Red: Cy3 labeled EpL1 conjugate
Green: actin
Blue: nucleus

IC50 = 21 nM
IC50 = 3597 nM

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EpL-trigger conjugates are internalized by human bronchial epithelial cells and reduce αENaC expression and activity.

Fully differentiated HBE cells in ALI culture

αENaC mRNA expression

Amiloride-sensitive current

Airway surface liquid volume

*Courtesy Matthias Salathe*
EpL-trigger conjugates are internalized by rat pulmonary epithelial cells in vivo following oropharyngeal (OP)delivery.
EpL-trigger conjugates silence lung αENaC expression in vivo

Rat whole lung αENaC mRNA expression

Epl1-trigger conjugate

Day 1: IT dose 2 mg/kg; Day 9 sacrifice

Relative expression

Vehicle Epl-trigger

-58%

Immunohistochemistry with αENaC antibody

Red: αENaC
Green: actin
Blue: nucleus

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EpL-trigger conjugates improve potency and uniformity of αENaC mRNA silencing in the lung, with durable reduction in target expression.

**Rat whole lung αENaC expression**

EpL2-trigger2 conjugate

Day 1-3: OP dose; Day 9 sacrifice

<table>
<thead>
<tr>
<th>Dose Level (mg/kg)</th>
<th>Relative expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1.5</td>
</tr>
<tr>
<td>0.1</td>
<td>1.0</td>
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<tr>
<td>0.2</td>
<td>0.5</td>
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<tr>
<td>0.3</td>
<td>0.5</td>
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<tr>
<td>0.4</td>
<td>0.5</td>
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<tr>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>0.7</td>
<td>0.5</td>
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- EpL-trigger

**Rat whole lung αENaC expression**

Day 1, 2: OP dose 0.7 mg/kg EpL2-trigger2

<table>
<thead>
<tr>
<th>Study day</th>
<th>Relative expression</th>
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<tbody>
<tr>
<td>0</td>
<td>0.00</td>
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<tr>
<td>10</td>
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<tr>
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<tr>
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<tr>
<td>40</td>
<td>1.00</td>
</tr>
<tr>
<td>50</td>
<td>1.25</td>
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</tbody>
</table>

Durable mRNA silencing supports every other week (or less frequent) dose regimens.
Aerosol inhalation improves delivery efficiency of EpL-αENaC RNAi trigger conjugates

- No changes in renal αENaC mRNA expression or serum potassium levels
- Well-tolerated, with no significant findings in clinical chemistry, hematology or histopathology
αENaC silencing in lung does not cause pulmonary edema

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Extravascular lung water index

**Rat whole lung αENaC expression**

*Day 1, 2: IT dose 4 mg/kg EpL1-trigger*  
*Day 5 sacrifice*

**Extravascular lung water index**

**Relative expression**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>EpL-trigger</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>0.67 ± 0.1</td>
</tr>
</tbody>
</table>

- 83%

**EVLWI (ml/kg)**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>EpL-trigger</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0</td>
<td>2.2 ± 0.1</td>
</tr>
</tbody>
</table>

*Course and evaluation of experimental pulmonary edema*
αENaC silencing does not exacerbate pulmonary edema or slow its resolution following oleic acid-induced lung injury

- Rats received IT EpL conjugate at dose that silenced >80% αENaC mRNA in lung
- Lung injury induced with IV oleic acid
- Monitor resolution of pulmonary edema over 48 hr post-injury
Sheep mucociliary clearance

**Mucociliary clearance measurements:** pre-dose baseline and Day 17
- Inhalation of aerosolized $^{99m}$Tc-labeled sulfur colloid
- Clearance measured via gamma imaging (5 min intervals over two hours)

**Group 1** (n=3): aerosolized EpL2-trigger2 conjugate
- 0.07 mg/kg deposited dose on Days 1-3

**Group 2** (n=2): aerosolized amiloride (3 mL 3 mM)
- 3 mL 3 mM immediately prior to MCC scan (1-2 hour effect in lung)

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Sheep mucociliary clearance
Amiloride administered immediately prior to scan

Presented at ERS International Congress 2018

Courtesy Juan Sabater
Sheep mucociliary clearance
EpL2-trigger2 conjugate administered 14-16 days prior to scan

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Sheep mucociliary clearance

% retention of baseline baseline
EpL2-trigger2 + 7%
amiloride + 22%

Courtesy Juan Sabater
Conclusions

• Inhaled EpL-αENaC RNAi trigger conjugates produce selective, durable, renal-sparing silencing of pulmonary αENaC expression

• Deep αENaC mRNA silencing in the lung does not cause, exacerbate or slow the resolution of pulmonary edema

• Improved mucociliary clearance is observed in sheep two weeks after inhalation of aerosolized conjugate

• ARO-ENaC for cystic fibrosis is Arrowhead’s first program to employ the pulmonary epithelial delivery platform

• The platform may be adapted to additional therapeutic targets in the pulmonary epithelium, particularly those that are currently inaccessible to traditional small molecule or antibody approaches
Acknowledgements

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