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

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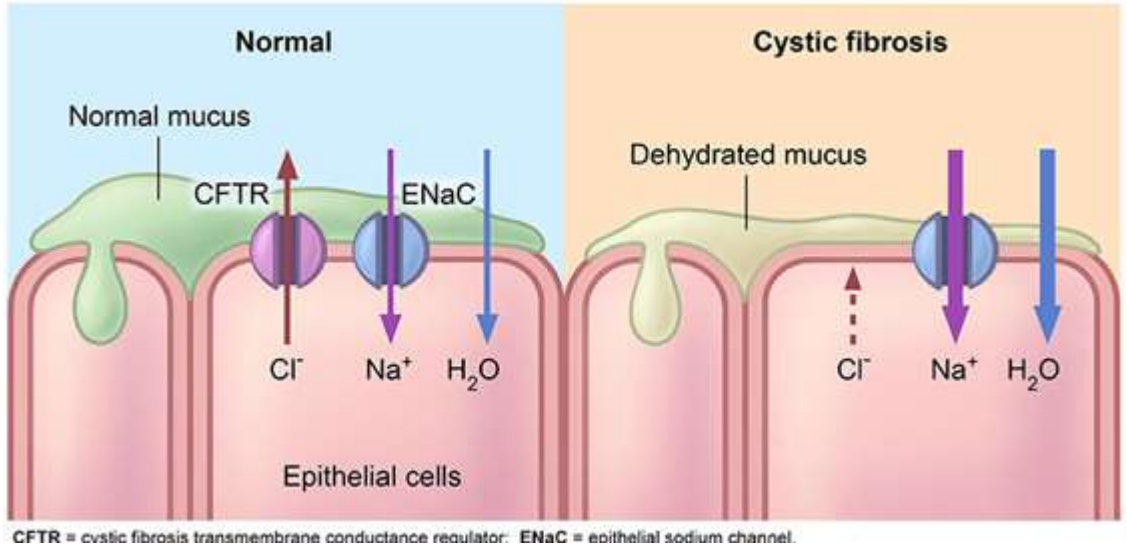
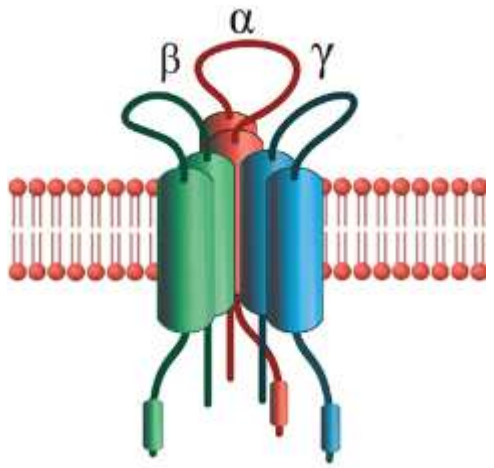
Disclosures

- I am an employee and shareholder of Arrowhead Pharmaceuticals, Inc.

Increased epithelial sodium channel (ENaC) activity promotes mucus dehydration in cystic fibrosis lung disease



CFTR = cystic fibrosis transmembrane conductance regulator; ENaC = epithelial sodium channel.



Bester-Meredith 2015

- 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

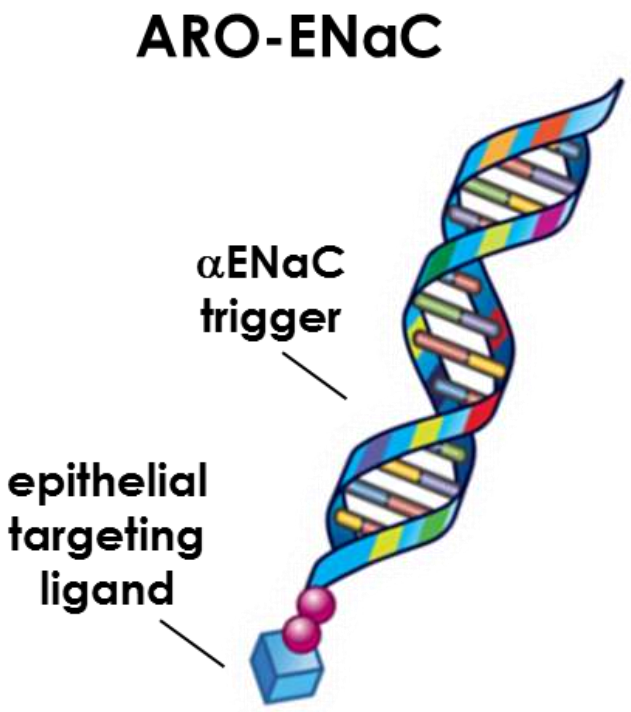
Acute Hyperkalemia Associated with Inhalation of a Potent ENaC Antagonist: Phase 1 Trial of GS-9411

Thomas G. O'Riordan, MD,[1] Karl H. Donn, PhD,[2] Peter Hodsman, MD,[3] John H. Ansede, PhD,[2] Terry Newcomb, PhD,[1] Sandra A. Lewis, MS,[1] William D. Flitter, PhD,[1] Vicki Shigekane White, BS,[1] M. Ross Johnson, PhD,[2] A. Bruce Montgomery, MD,[4] David G. Warnock, MD,[5] and Richard C. Boucher, MD[6]

"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

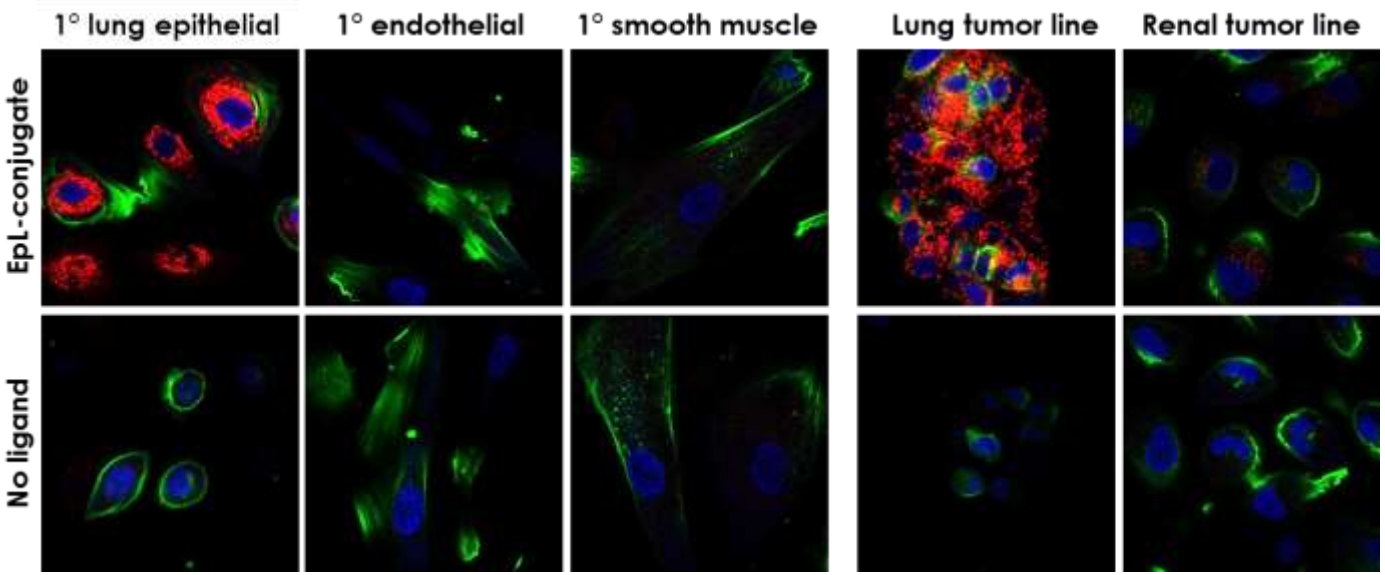
TRiM™ platform: Targeted RNAi Molecules



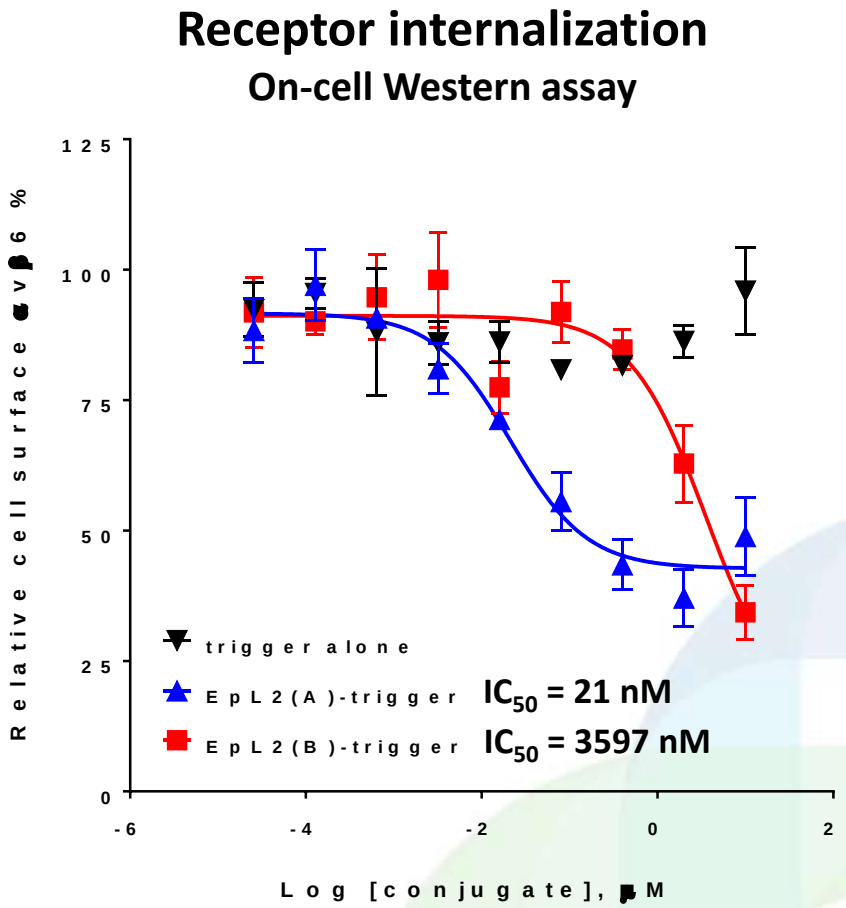
EpL = integrin αvβ6 ligand

- 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

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

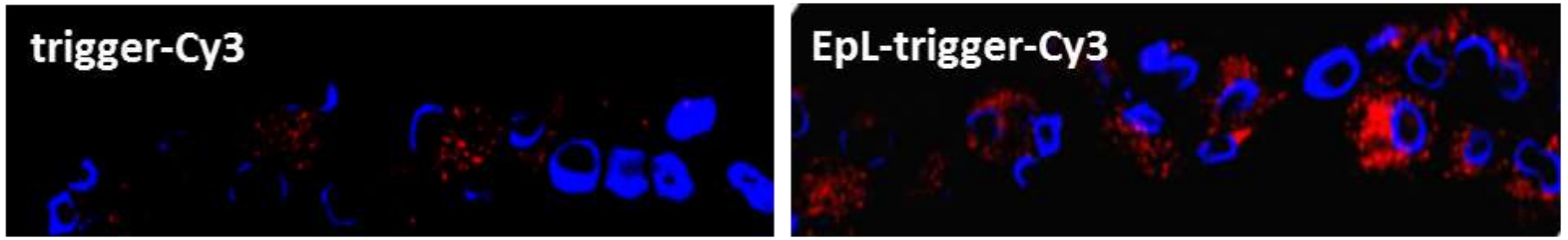


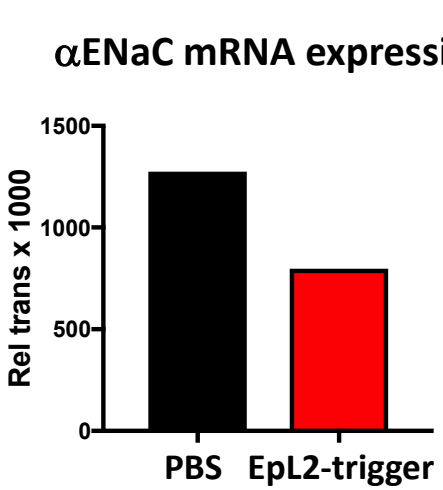
Red: Cy3 labeled EpL1 conjugate
Green: actin
Blue: nucleus

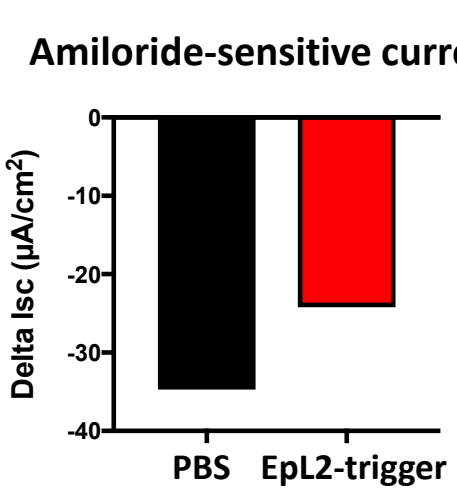


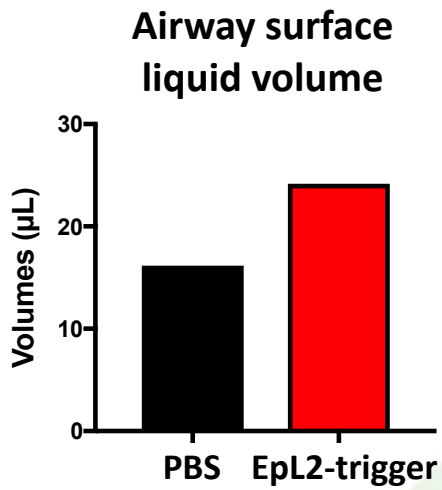
EpL-trigger conjugates are internalized by human bronchial epithelial cells and reduce αENaC expression and activity

Fully differentiated HBE cells in ALI culture



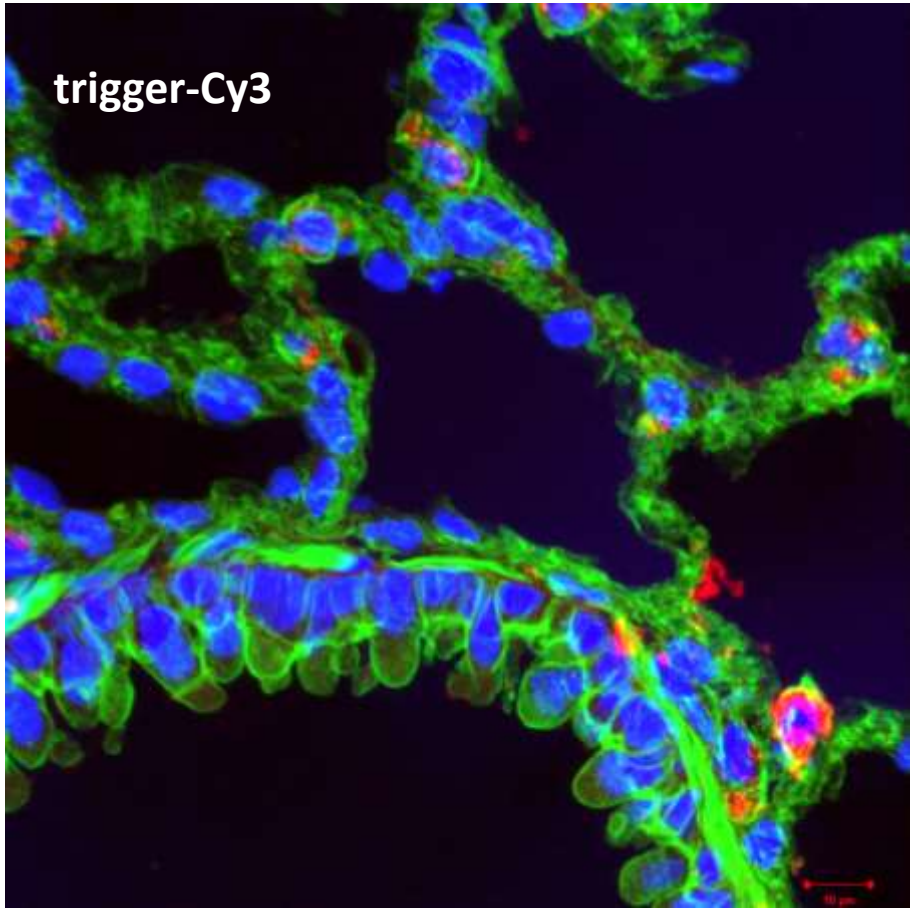


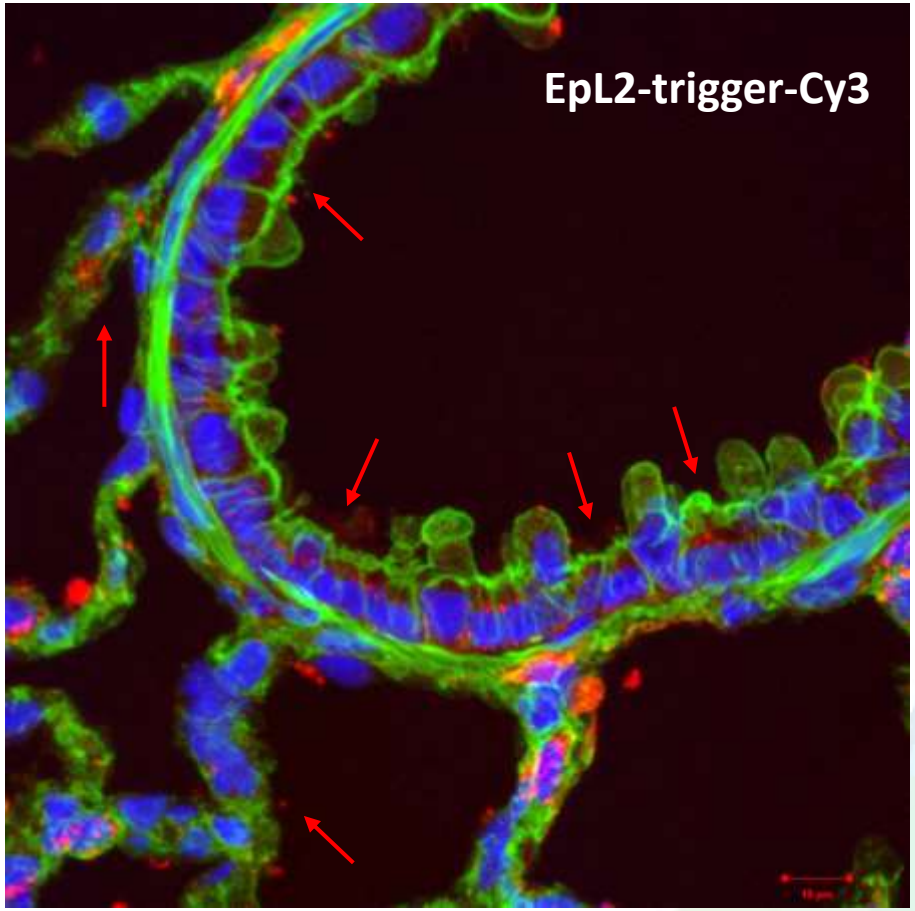




Courtesy Matthias Salathe

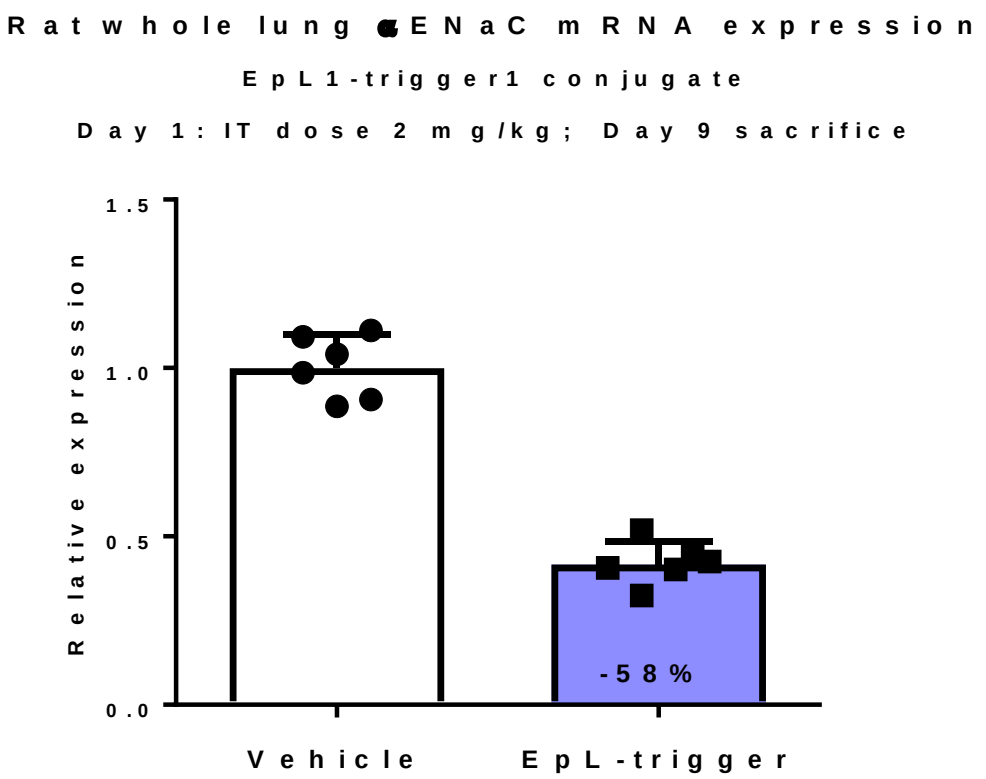
EpL-trigger conjugates are internalized by rat pulmonary epithelial cells *in vivo* following oropharyngeal (OP)delivery



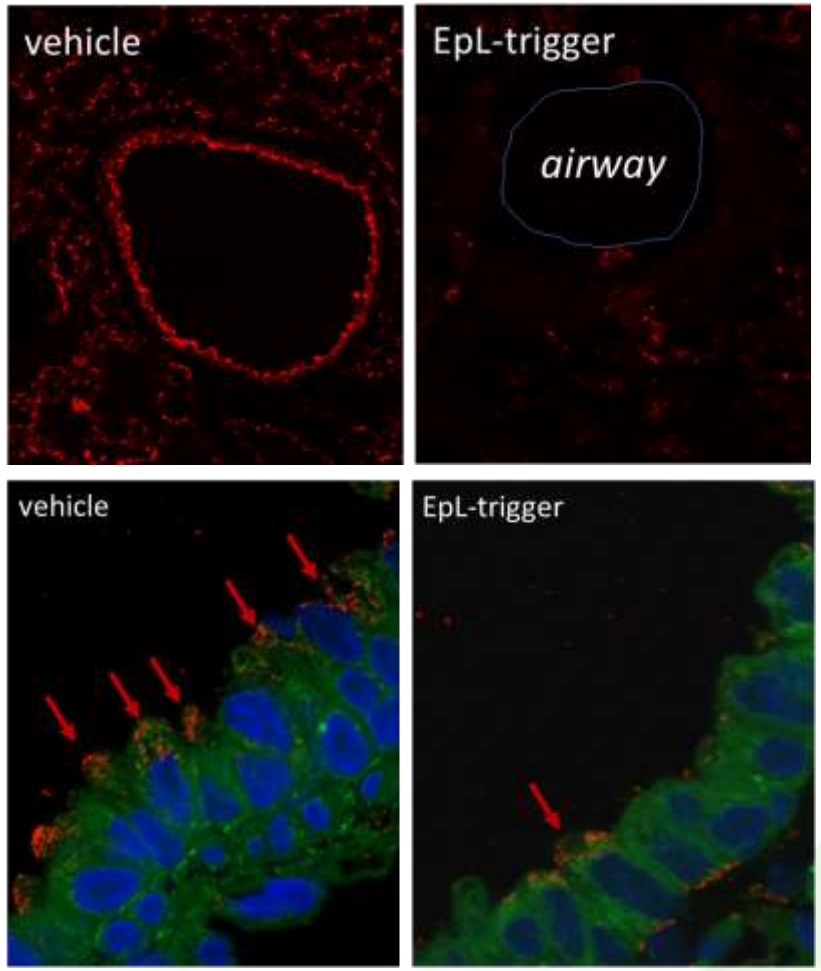


Red: Cy3 labeled EpL2 conjugate
Green: actin
Blue: nucleus

EpL-trigger conjugates silence lung αENaC expression *in vivo*

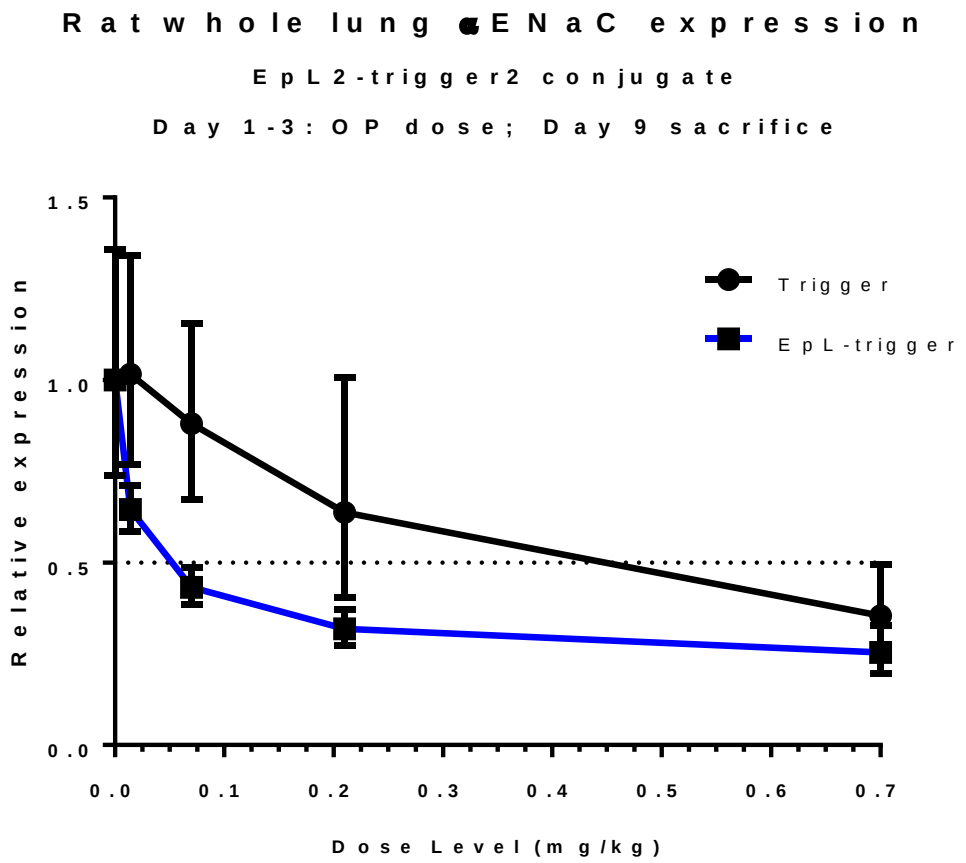


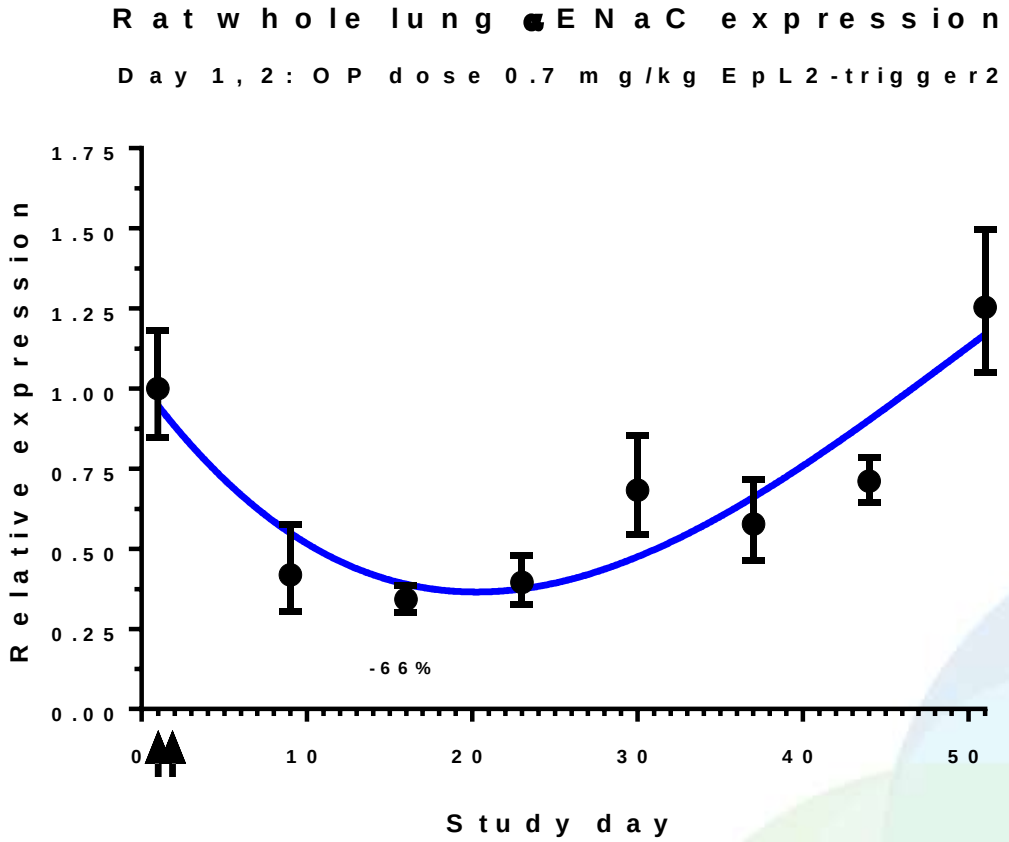
Immunohistochemistry with αENaC antibody



Red: αENaC
Green: actin
Blue: nucleus

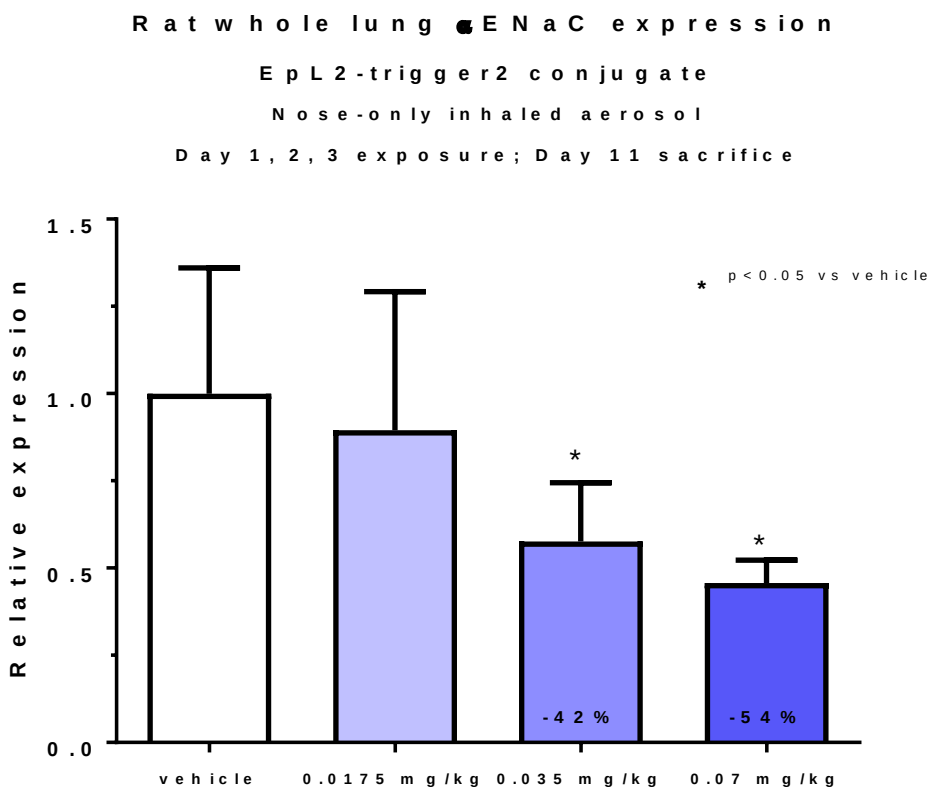
EpL-trigger conjugates improve potency and uniformity of αENaC mRNA silencing in the lung, with durable reduction in target expression

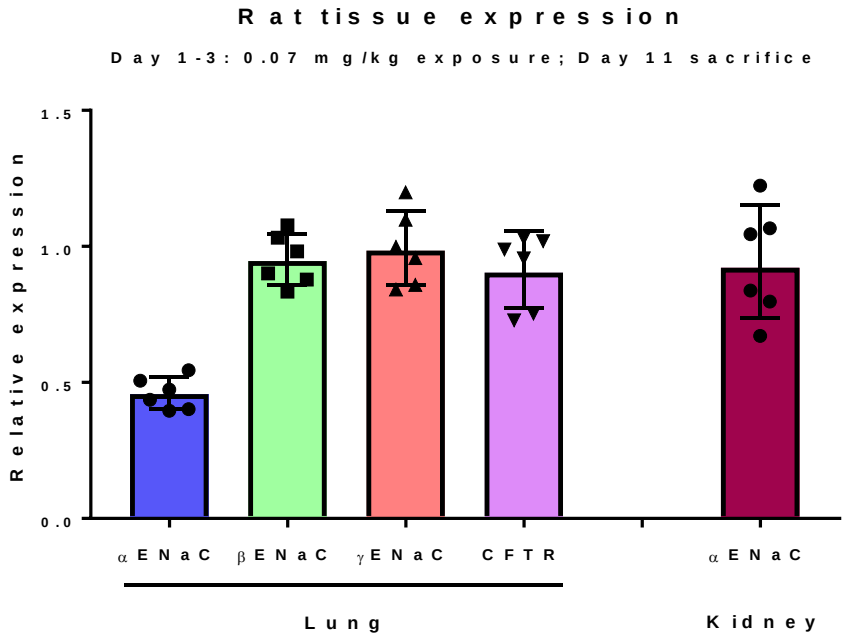




Durable mRNA silencing supports every other week (or less frequent) dose regimens

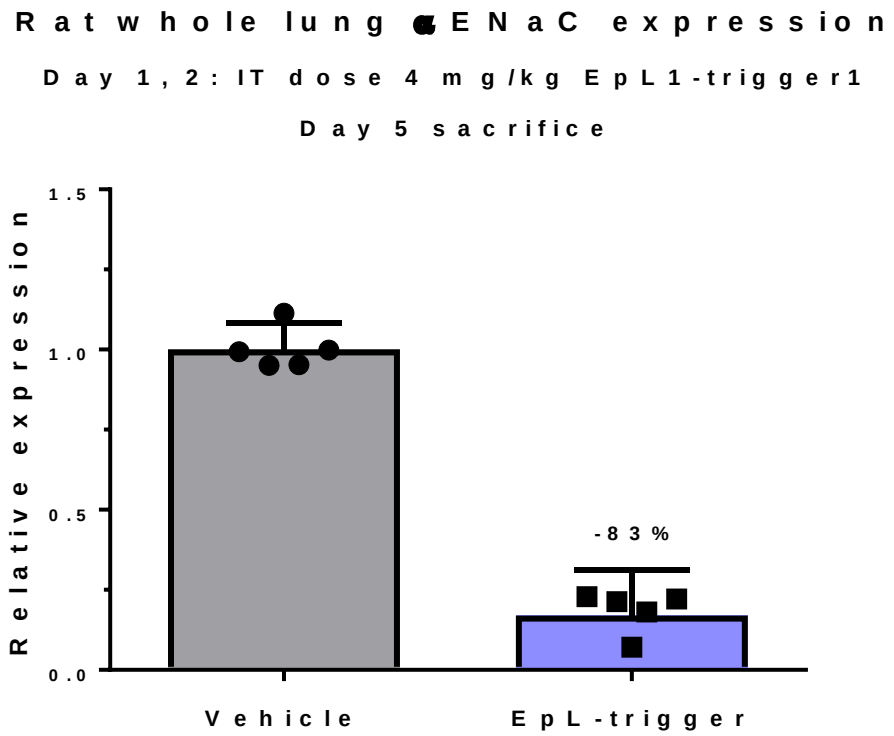
Aerosol inhalation improves delivery efficiency of EpL-αENaC RNAi trigger conjugates

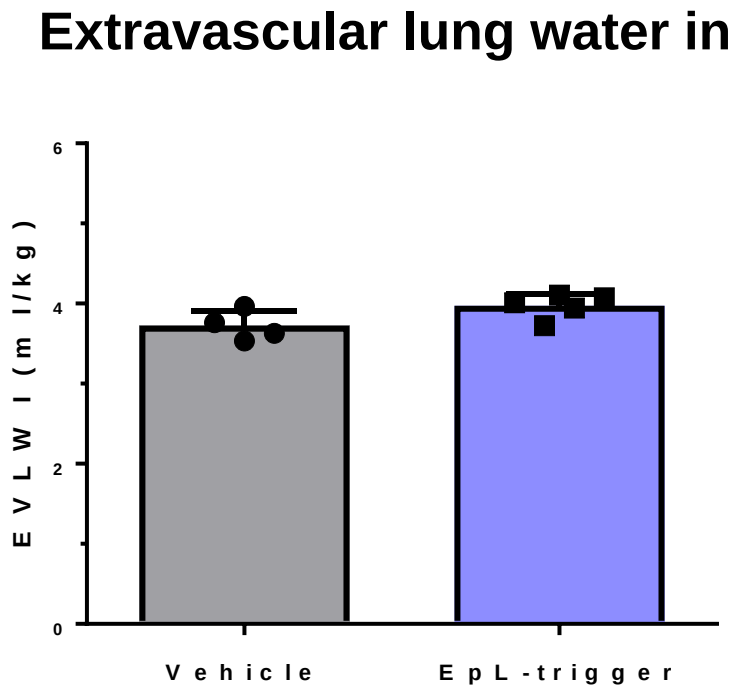




- No changes in renal αENaC mRNA expression or serum potassium levels
- Well-tolerated, with no significant findings in clinical chemistry, hematology or histopathology

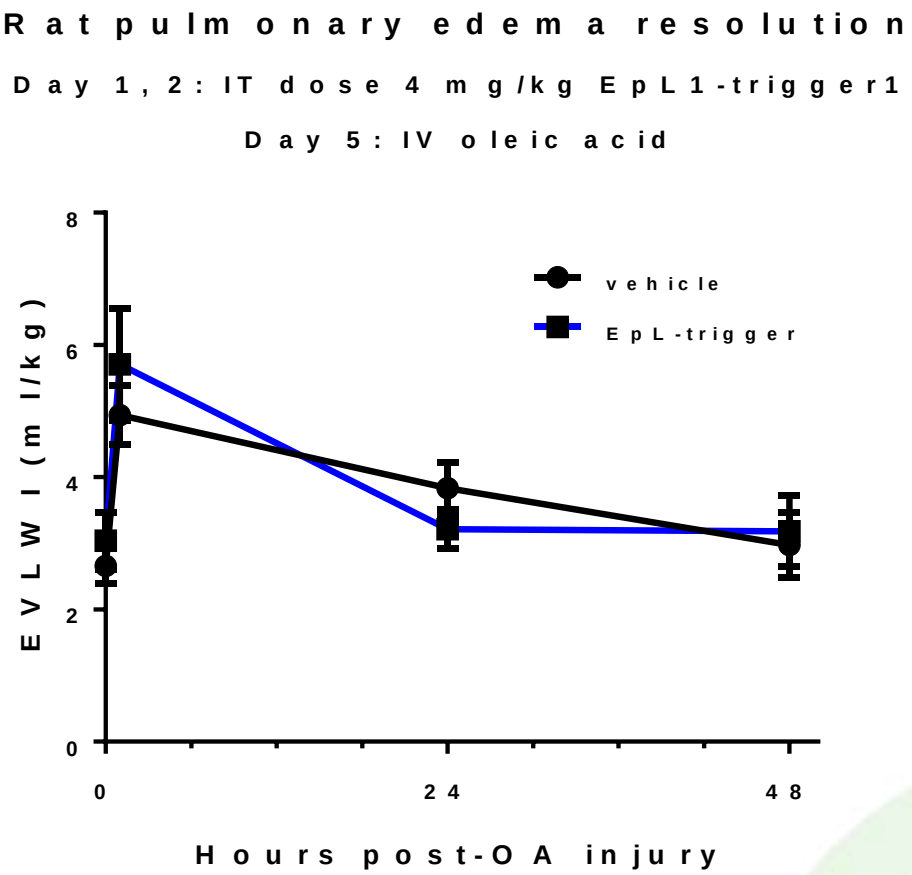
αENaC silencing in lung does not cause pulmonary edema





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

Mucocilary 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

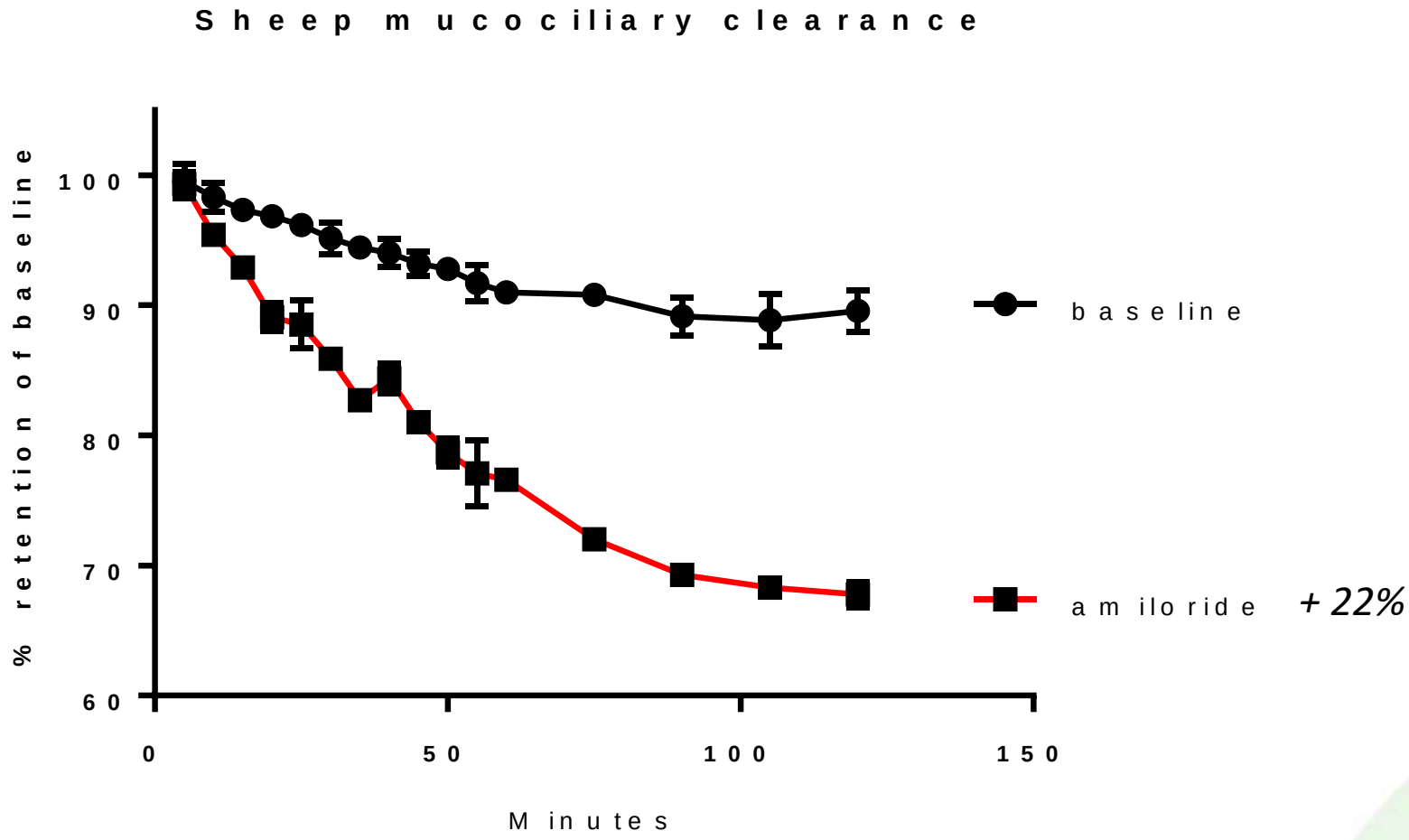
- 0.07 mg/kg deposited dose on Days 1-3

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)

Sheep mucociliary clearance

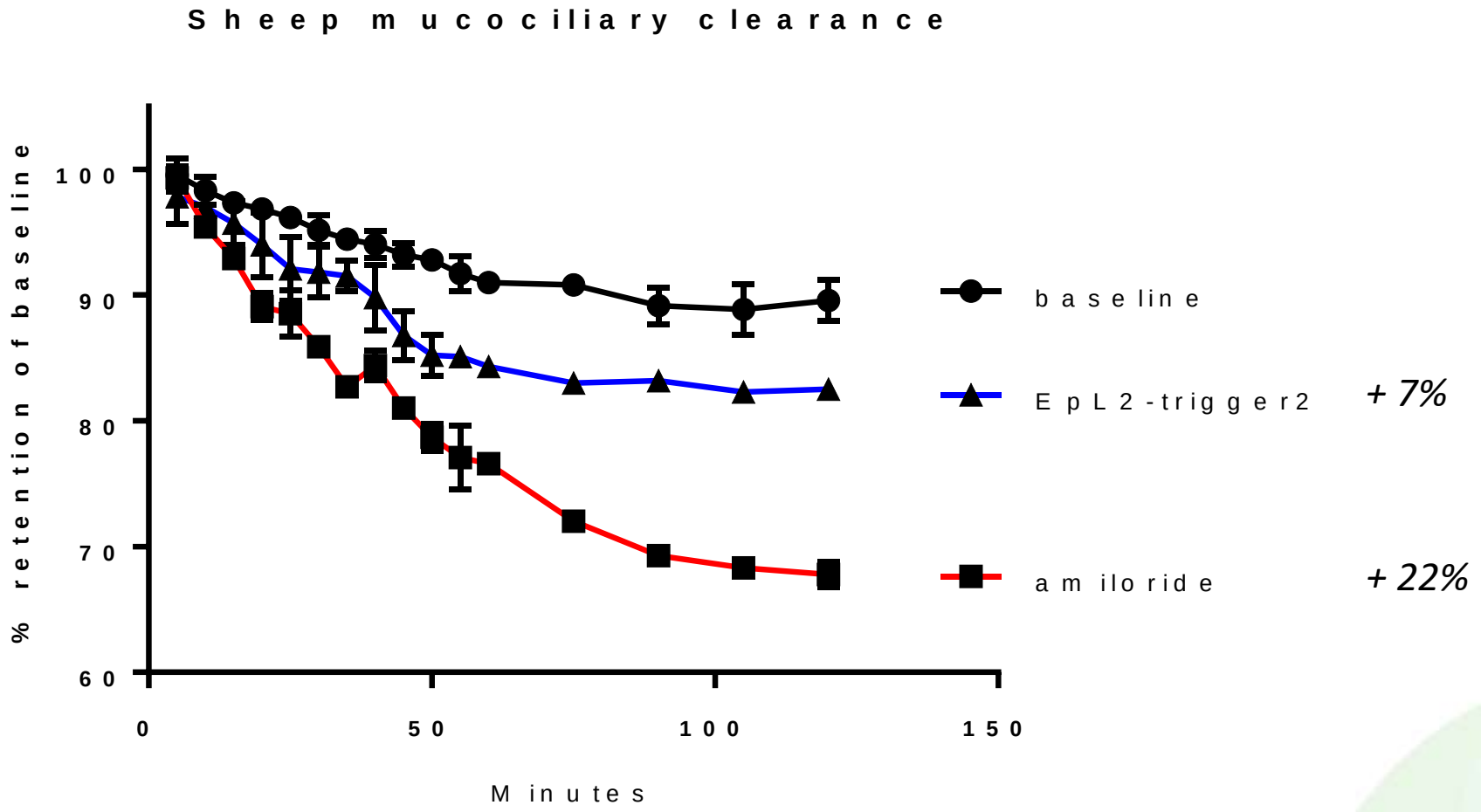
Amiloride administered immediately prior to scan



Courtesy Juan Sabater

Sheep mucociliary clearance

EpL2-trigger2 conjugate administered 14-16 days prior to scan



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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