A Lung-Targeted RNAi Therapeutics Delivery Platform Compatible with Subcutaneous (s.c.) Administration Mediates Pulmonary RAGE Silencing in Rodents and Nonhuman Primates

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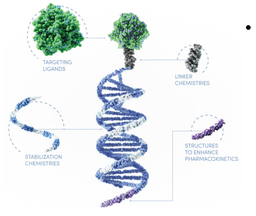
Rationale



- Arrowhead's pulmonary Targeted RNAi Molecule (TRiM™) delivery platform facilitates selective delivery of therapeutic siRNAs (RNAi triggers) to the lung epithelium via an integrin $\alpha_v\beta_6$ targeting moiety, mediating durable gene silencing upon inhalation.

- Three clinical candidates utilizing the inhaled pulmonary TRiM™ delivery platform have recently entered Phase 1/2a trials: ARO-RAGE for pulmonary inflammation, ARO-MUC5AC for muco-obstructive lung disease and ARO-MMP7 for pulmonary fibrosis.
- While inhaled dosing offers a convenient local delivery solution, systemic administration via subcutaneous (s.c.) injection would offer additional flexibility for some therapeutic applications.
- Here, we demonstrate that the inhaled pulmonary TRiM™ delivery platform is also compatible with s.c. administration and is capable of effectively silencing lung expression of the receptor for advanced glycation end-products (RAGE) in rodents and non-human primates.

Methods

- Rats received weekly s.c. injections of vehicle (saline) or integrin-targeted siRNA conjugate specific for rat *RAGE* mRNA. Serum samples were collected weekly and assayed for soluble RAGE (sRAGE), a proteolytic product of lung-expressed RAGE shed into circulation and a biomarker of target engagement in the lung (R&D Systems). Lung tissue *RAGE* mRNA expression was determined by RT-qPCR.
- Rats received s.c. injections of RAGE siRNA conjugated to integrin targeting ligand, siRNA alone, or siRNA conjugated to an inactive enantiomer of the integrin targeting ligand. A modified non-silencing version of the RAGE siRNA incapable of loading into the RISC complex was included as an additional negative control.
- Plasma siRNA concentrations were measured via LCMS and tissue siRNA exposure visualized by in situ hybridization with a probe complementary to the antisense siRNA strand (miRNAScope, ACDBio).
- In non-human primate (NHP) studies, cynomolgus monkeys received weekly s.c. injections of vehicle (saline) or ARO-RAGE (currently in Phase 1/2a clinical trials). Serum samples were collected weekly, and sRAGE target engagement biomarker measured by Gyros immunoassay with an antibody that recognizes the human protein.

Lung RAGE silencing via s.c. route in the rat

Lung *RAGE* mRNA expression a week after 1 s.c. dose of siRNA conjugate

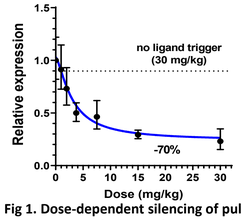


Fig 1. Dose-dependent silencing of pulmonary *RAGE* mRNA one week after a single s.c. injection, relative to vehicle controls. RNAi trigger alone lacking integrin targeting ligand (dotted line) minimally silenced lung RAGE expression when delivered via s.c. route.

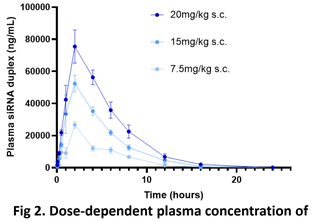


Fig 2. Dose-dependent plasma concentration of siRNA duplex after single subcutaneous injection.

Targeting ligand binding to $\alpha_v\beta_6$ receptor is required for effective pulmonary gene silencing via s.c. route

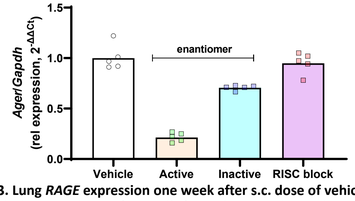
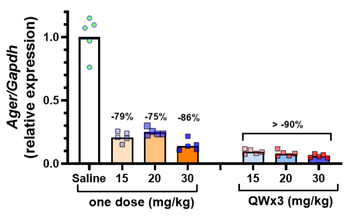


Fig 3. Lung *RAGE* expression one week after s.c. dose of vehicle or RNAi trigger conjugate (15 mg/kg). An siRNA conjugate incorporating the receptor non-binding (inactive) enantiomer of the targeting ligand was significantly less effective than the high affinity (active) targeting ligand enantiomer. A control RAGE siRNA conjugate modified to prevent antisense strand incorporation into the RISC complex did not silence lung *RAGE* mRNA.

Lung *RAGE* mRNA expression a week after 1 or 3 weekly s.c. doses



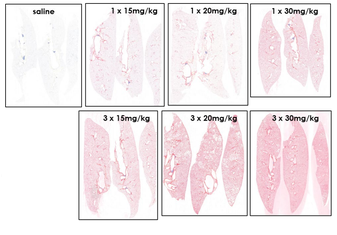
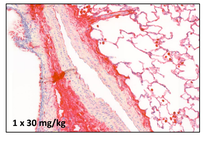
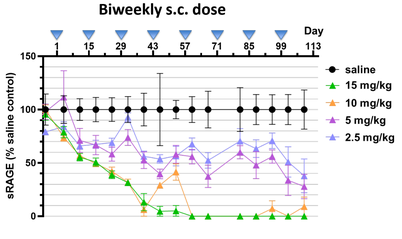


Fig 4. Lung *RAGE* mRNA expression a week after 1 or 3 weekly s.c. doses of RNAi trigger conjugate (left graph). Lung tissue siRNA exposure via ISH (middle panels).



After s.c. dosing, siRNA is uniformly distributed in lung tissue and the perivascular compartment. Uptake of siRNA was observed in multiple lung cell types including airway epithelium, alveolar epithelium and pulmonary macrophages.

Serum sRAGE response to biweekly or monthly s.c. doses of siRNA conjugate



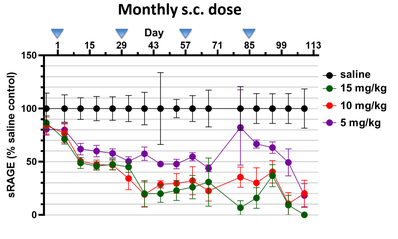


Fig 5. Serum sRAGE (lung target engagement biomarker) is fully depleted (below LLOQ) after biweekly s.c. injection of 10-15 mg/kg RNAi conjugate. Maintenance of sRAGE depletion can be achieved with monthly 10-15 mg/kg s.c. dosing.

Lung RAGE silencing via s.c. route in NHP

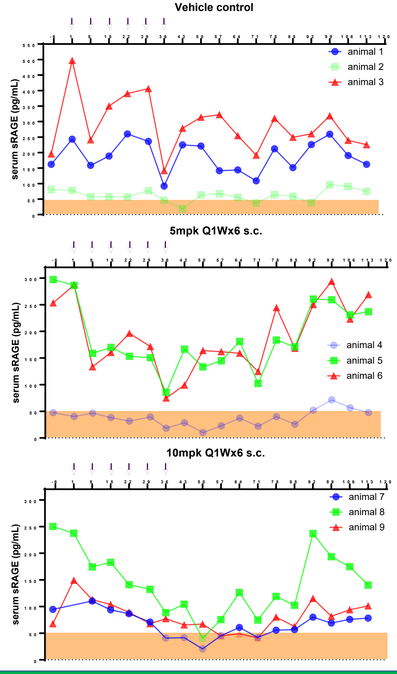


Fig 6. Cynomolgus monkeys received six weekly s.c. injections of vehicle or ARO-RAGE (5 mg/kg or 10 mg/kg) with serial serum samples collected for quantitation of sRAGE target engagement biomarker (lower limit of quantitation 50 pg/mL marked orange). Baseline serum sRAGE expression for one animal in group was near the LLOQ.

Individual saline vehicle control animals (n=3) show relatively stable serum sRAGE expression over time.

Animals receiving 5 mg/kg s.c. ARO-RAGE (n=3) showed reduced serum sRAGE within a week of the first injection, reaching nadir after five doses. Upon cessation of dosing, serum sRAGE levels began recovering approximately five weeks later. Baseline serum sRAGE expression for one animal in group was near the LLOQ.

One animal receiving 10 mg/kg s.c. ARO-RAGE showed substantially reduced serum sRAGE beginning a week after the first dose and continuing to decline from 250 pg/mL to near the LLOQ two weeks after the sixth weekly dose (Day 50, with expression recovering approximately seven weeks after the last dose. Baseline serum sRAGE expression for the other two animals in the group was near the LLOQ.

Arrowhead's pulmonary Targeted RNAi Molecule (TRiM™) platform is compatible with inhaled or subcutaneous routes of administration, potentially offering alternative dose forms for the delivery of therapeutic siRNAs to the lung.