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A Clinical-Stage Inhaled RNAi Therapeutic For Pulmonary Inflammation Mediates Durable RAGE Silencing in Nonhuman Primates (NHP)

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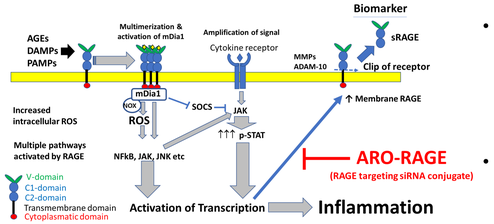
Introduction

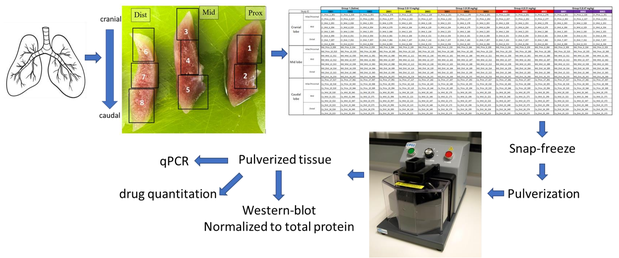
- The Receptor for Advanced Glycation-End products (RAGE) recognizes diverse pro-inflammatory ligands, is highly expressed in type 1 alveolar cells and is further upregulated by inflammation.
- Full-length membrane RAGE (mRAGE) amplifies and sustains chronic inflammation by promoting downstream of NFκB- or STAT-dependent signaling but has been challenging to effectively be a drug target with traditional small molecule approaches.
- Soluble RAGE (sRAGE, a proteolytic cleavage product of mRAGE) is shed into the airway and serum and can be used as biomarker to estimate expression of full-length receptor in the lung.
- ARO-RAGE is a clinical stage inhaled RNAi therapeutic designed to silence pulmonary *RAGE* mRNA.
- Objective: evaluate the pharmacodynamic activity of inhaled ARO-RAGE in nonhuman primates



- ARO-RAGE utilizes Arrowhead's pulmonary TRiM™ (Targeted RNAi Molecule) delivery platform, which facilitates targeting ligand-mediated epithelial uptake of therapeutic siRNAs.
- Phase 1/2a clinical trials of ARO-RAGE are currently in progress.

Methods

- Male cynomolgus monkeys received aerosolized ARO-RAGE or vehicle (saline) through an endotracheal tube (ET) and mechanical ventilation or via mask in conscious animals.
- Serial serum and bronchoalveolar lavage (BAL) samples were collected to assess soluble RAGE (sRAGE) protein via Gyrolab™ Immunoassay (Gyros Protein Technologies) with a labelled antibody (R&D Systems, goat anti-human RAGE, polyclonal).
- Lung tissues were collected following humane euthanasia and samples processed for RNA isolation, siRNA drug and protein quantitation.

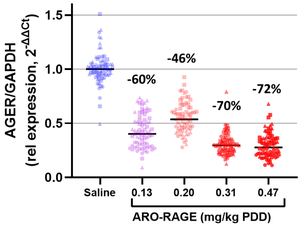


Dose-response of single inhaled dose of ARO-RAGE

1. Study design

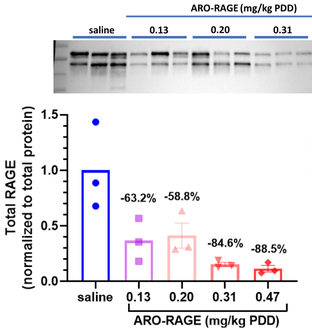
Three animals per group (exposure level)
Day 1: intubation, aerosol delivery of saline or ARO-RAGE
Day -7 (predose baseline), 15, 29: serum and BAL collections
Day 29: Euthanasia, tissue collection

2. Dose-dependent *RAGE* mRNA silencing in NHP lung



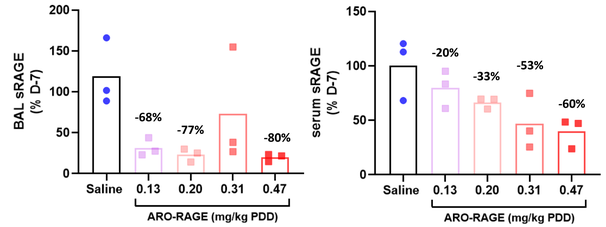
Dose-response of ARO-RAGE in silencing pulmonary *RAGE* (*AGER*) mRNA expression assessed via qPCR. Data from three animals per group, three right lobes per animal, three slice region and samples combined. Four weeks post-dose, a single pulmonary deposited dose (PDD) of 0.31 mg/kg ARO-RAGE or greater silenced approximately 70% of *RAGE* mRNA in the NHP lung.

3. A single 0.47 mg/kg inhaled dose of ARO-RAGE silences over 80% lung mRAGE protein at 4 weeks



Lung tissue mRAGE protein expression was assessed via Western analysis with an antibody that recognizes human RAGE (Abcam cat# Ab216239). Densitometry was performed with an iBlot instrument (Invitrogen) and normalized to total protein.

4. Dose-dependent reductions in target engagement biomarker sRAGE are detected in BAL and serum samples at 4 weeks



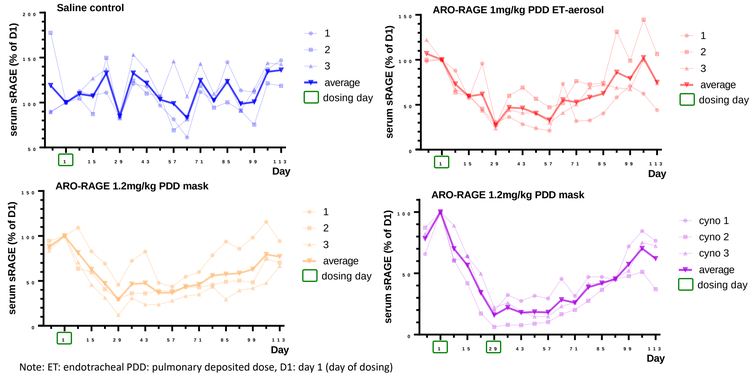
BAL and serum sRAGE protein expression 4 weeks post-dose (normalized to Day -7 predose baseline values). Reduced BAL sRAGE protein was observed at all dose levels (maximum reduction of 80% at 0.47 mg/kg PDD). Reductions in serum sRAGE were dose-dependent (maximum reduction of 60% at 0.47 mg/kg PDD).

Duration of BAL and serum sRAGE depletion after inhaled ARO-RAGE

1. Study design

Exposure via ET tube or mask (four groups; three animals per group)
Day 1: aerosol delivery of saline or ARO-RAGE (target 1 mg/kg exposure)
Weekly: serum collections to Day 113 (BAL collections at time points shown in the figure)
Day 29: second ARO-RAGE dose via mask (group 4 only)

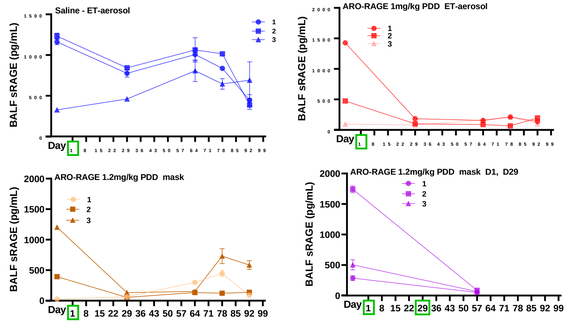
2. Serial serum sRAGE measurements after ARO-RAGE inhalation



Upper panels: Groups receiving aerosolized saline vehicle control (upper left) or ARO-RAGE (upper right) via ET tube.
Lower panels: Groups receiving either one dose (lower left) or two doses (lower right) of ARO-RAGE (lower left) via mask in conscious animals.

A single inhaled dose of ARO-RAGE reduced serum sRAGE by greater than 50%. A second dose of ARO-RAGE at Day 29 maintained deeper reductions of serum sRAGE for over a month.

3. Serial BAL sRAGE measurements after ARO-RAGE inhalation



BAL sRAGE is generally stable over time in animals exposed to saline vehicle (upper left).

Deep reductions in BAL sRAGE were observed in animals exposed to a single dose of ARO-RAGE via ET tube (upper right) or mask (lower left) and were maintained for over a month post-dose.

A second dose of ARO-RAGE, administered on Day 29 (lower right) reduced BAL sRAGE below the assay's limit of detection in Day 57 samples.

Note: ET: endotracheal PDD: pulmonary deposited dose, D1: day 1 (day of dosing)

- Inhaled ARO-RAGE produces dose-dependent, deep and durable reduction of RAGE expression in the NHP lung
- Measurement of BAL or serum sRAGE offers a novel biomarker approach to monitor ARO-RAGE target engagement in the lung