ARO-RAGE: Pre-Clinical and Clinical Overview

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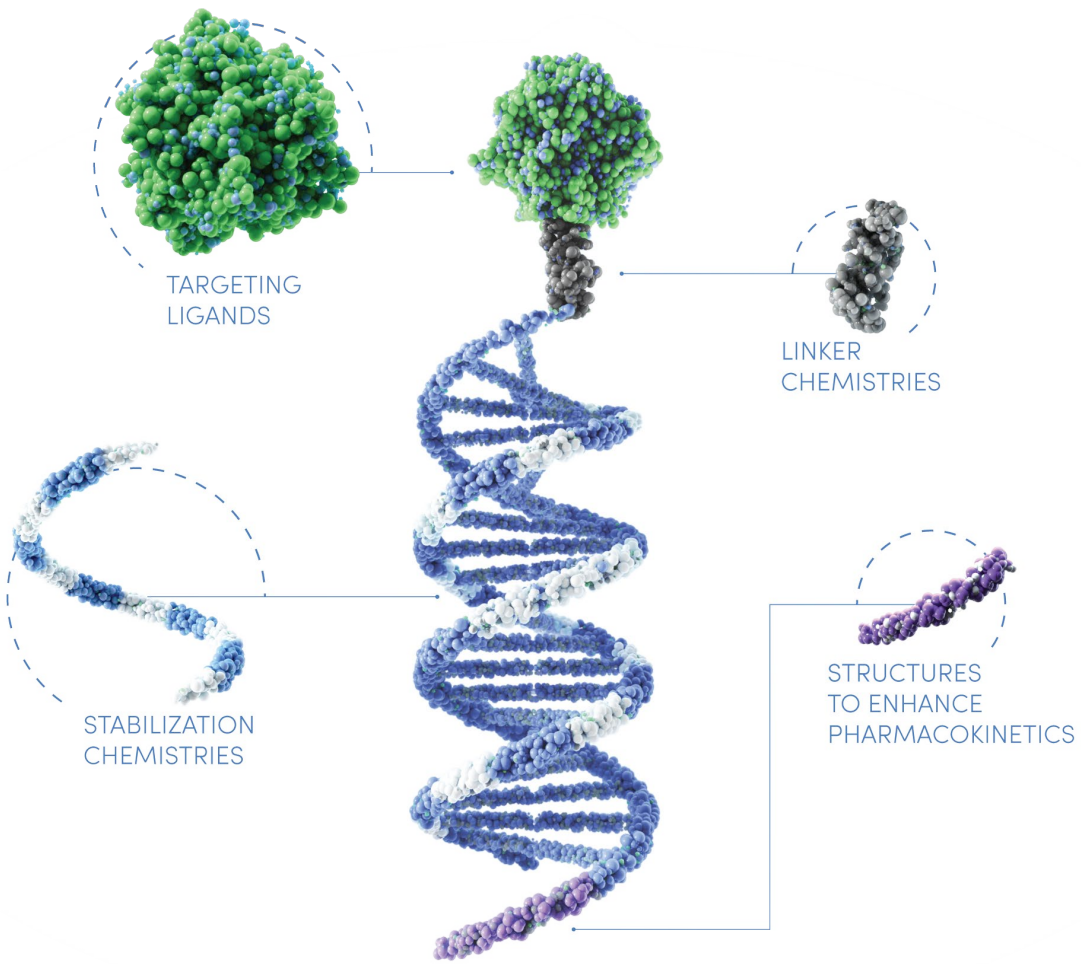
TRiM™ Platform for Pulmonary Delivery



Algorithmic Approach to Sequence Design and Selection

- Avoid microRNA and off-target knockdown while maximizing on-target activity
- Enhanced focus on early compound screening in non-GLP inhaled tox studies

Enhanced Modification Chemistry

- Maximize depth and duration of knockdown, minimize dose frequency

avβ6 Integrin Small Molecule Targeting Ligand Drives Epithelial Cell Uptake

- Increases potency of inhaled RNAi triggers
- Preferential delivery to epithelium over macrophage
- Transient receptor internalization
- No evidence of integrin receptor pharmacology

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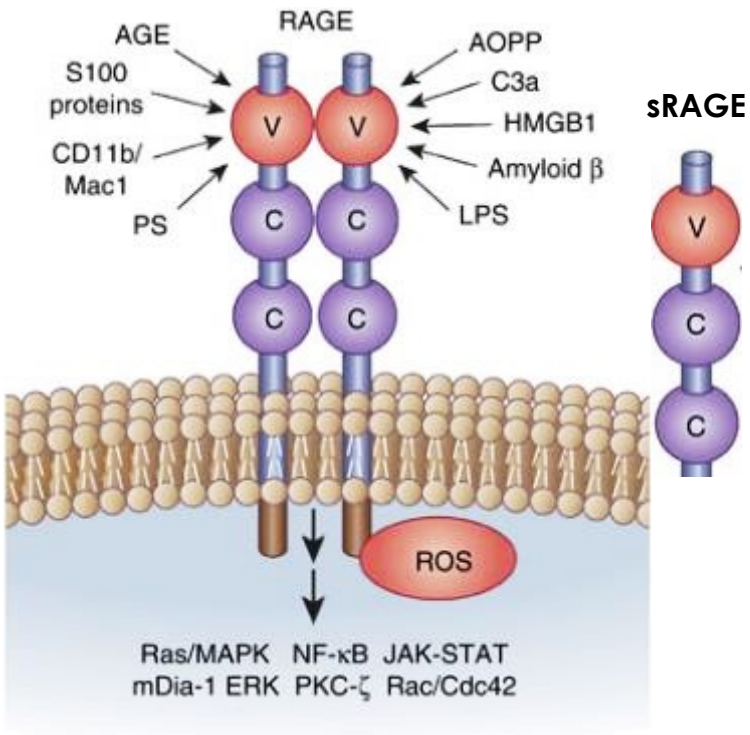
RAGE Regulates Airway Inflammation in Asthma

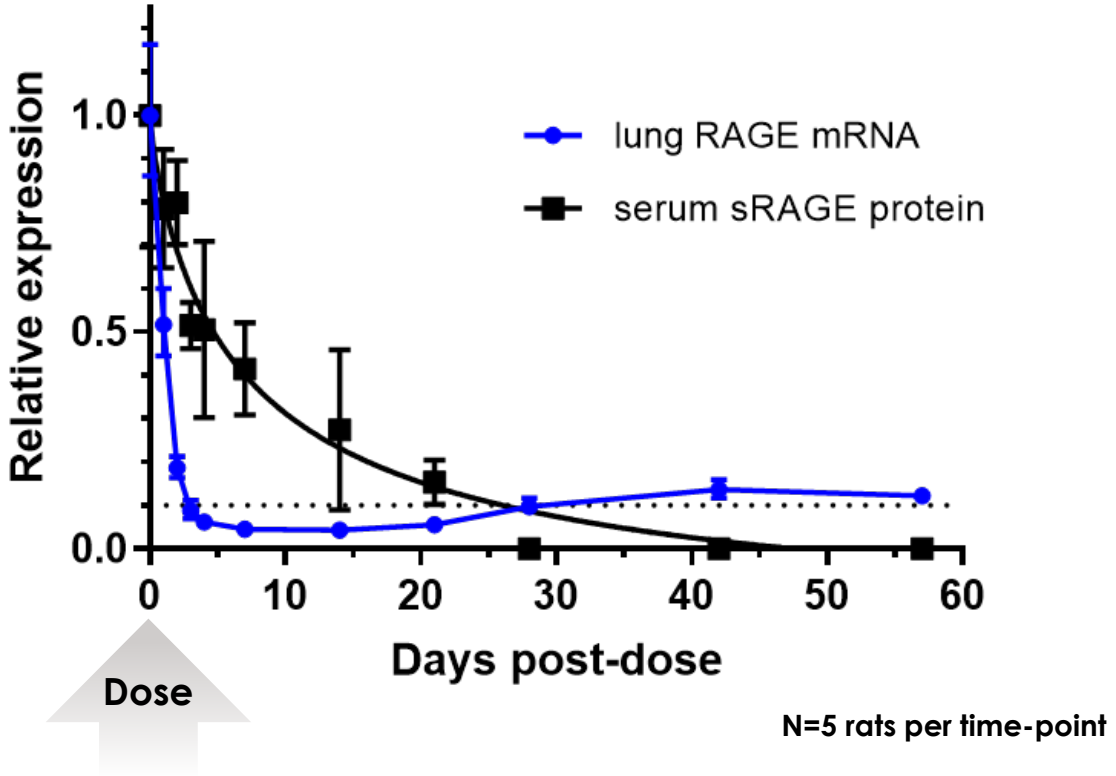
- The receptor for advanced glycation end-products (RAGE) is a pattern recognition receptor expressed abundantly in pulmonary epithelium, with low extrapulmonary expression.
- RAGE binds to a wide range of pro-inflammatory ligands, including HMGB1, S100 proteins, SAA, HSP70, and AGEs, resulting in activation of signalling pathways including NFκB and STAT6.[1]
- Animal models of asthma implicate RAGE as an upstream mediator of key Type-2 and non-Type-2 inflammatory cascades:
  - RAGE is required for allergen-induced release of IL-33, accumulation of ILC2s, and upregulation of IL-5 and IL-13.[2]
  - Models of severe steroid resistant neutrophilic airway disease indicate that inflammasome activation and neutrophil accumulation are RAGE-dependent.[3]
- RAGE can be cleaved to generate soluble RAGE (sRAGE), which is secreted into the airway and into serum.



1. Perkins TN et al. Allergy 2020.
2. Oczypok EA et al. J Allergy Clin Immunol 2015.
3. Killian KN et al. Front Immunol 2023.
4. Image: Yamamoto Y et al. Kidney Int 2012.

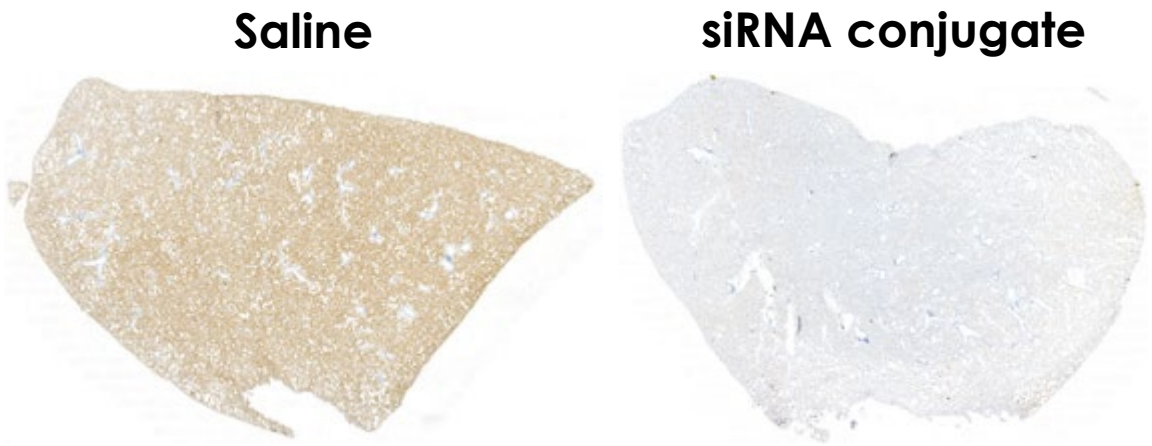
Deep and Sustained Lung RAGE Silencing After Single Inhaled Dose of siRNA Conjugate in Rats

Rat RAGE Expression After 0.5 mg/kg Inhaled Deposited Dose – siRNA Conjugate



N=5 rats per time-point

Day 36 Lung RAGE Protein IHC

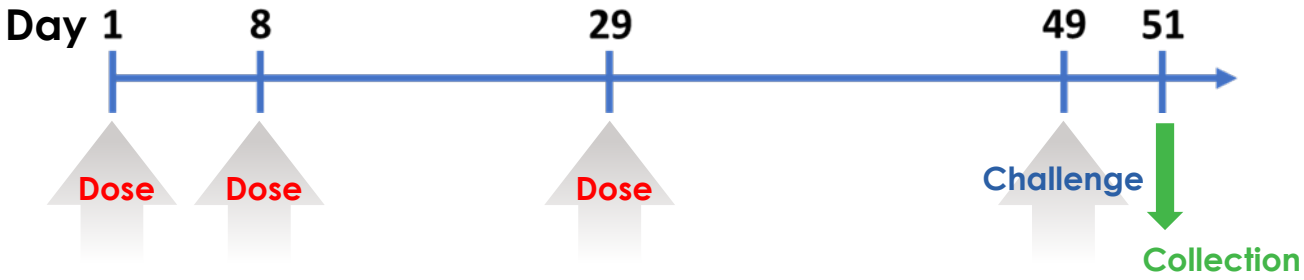


Kasahara, et al, A5013, *ATS* 2022

IHC = immunohistochemistry
sRAGE = soluble RAGE

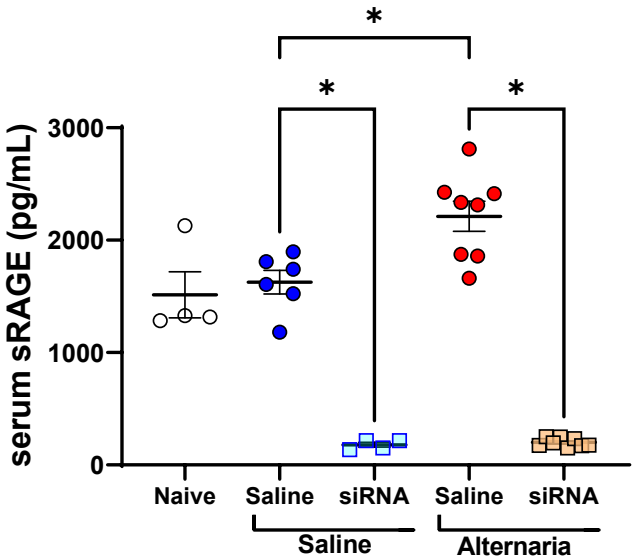
RAGE-Directed siRNA Conjugate Prevented Allergen-Induced Upregulation of sRAGE in Rat Model of Allergic Asthma

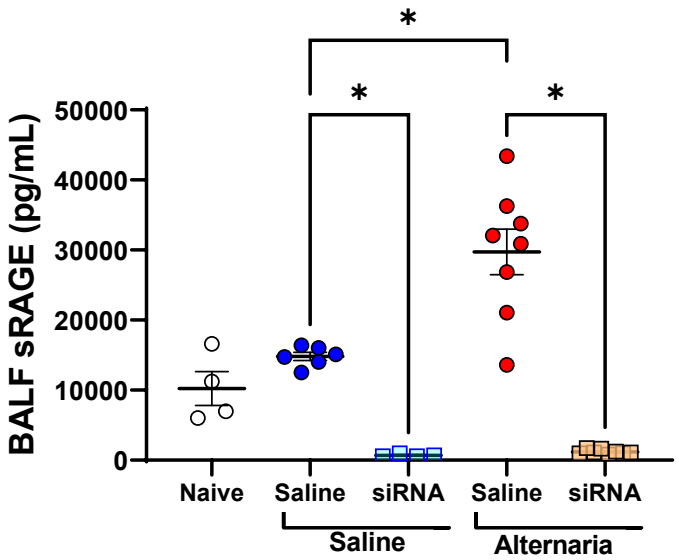
Brown-Norway rats (n=4-8/group)



Dose siRNA (3 mg/kg, IT)

Challenge *Alternaria alternata* extract (400 µg, IT)





*p<0.05

BALF: bronchoalveolar lavage fluid
IT: intratracheal
sRAGE: soluble RAGE

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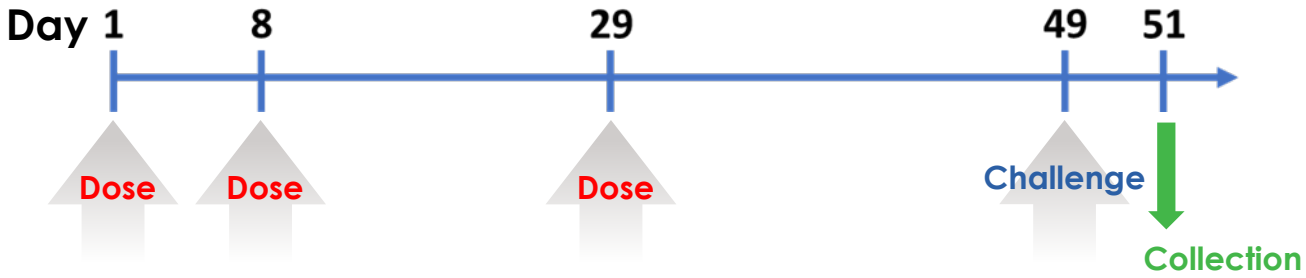
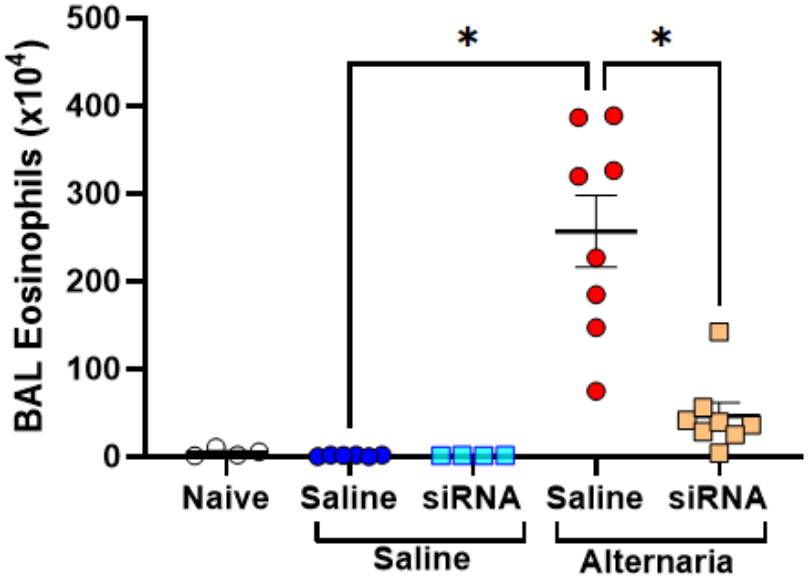
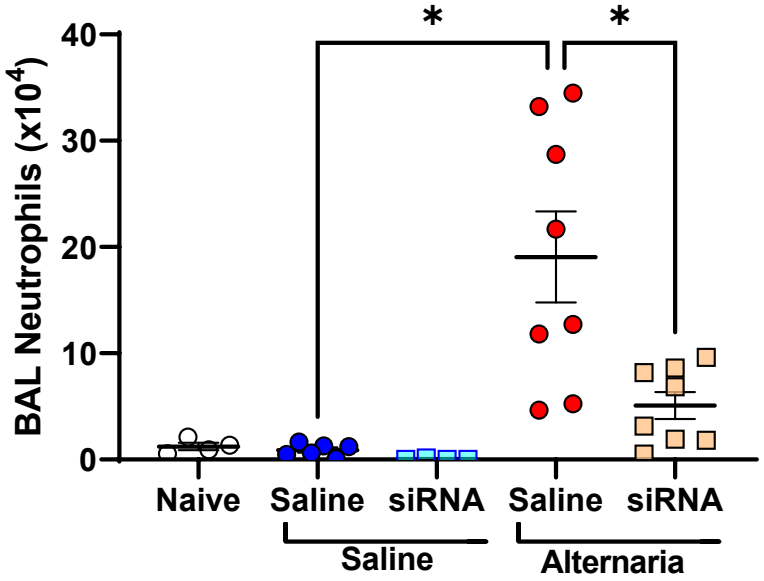
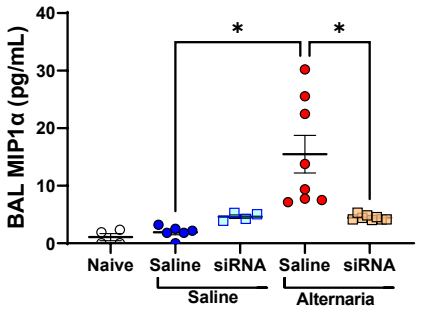
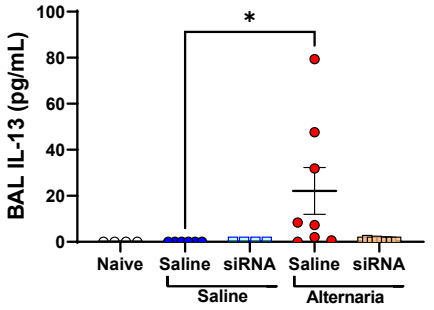
Deep RAGE Silencing Limited Inflammation in Rat Model of Allergic Asthma

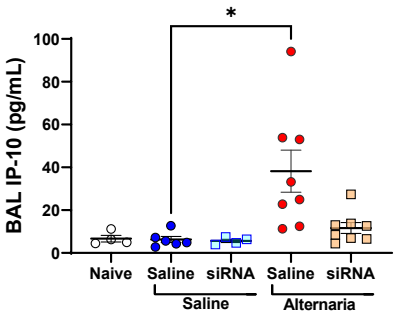












RAGE Silencing Reduced Inflammatory Cell Recruitment and Cytokines

*p<0.05
Alt: alternaria
BAL: bronchoalveolar lavage
NS: normal saline
Veh: vehicle

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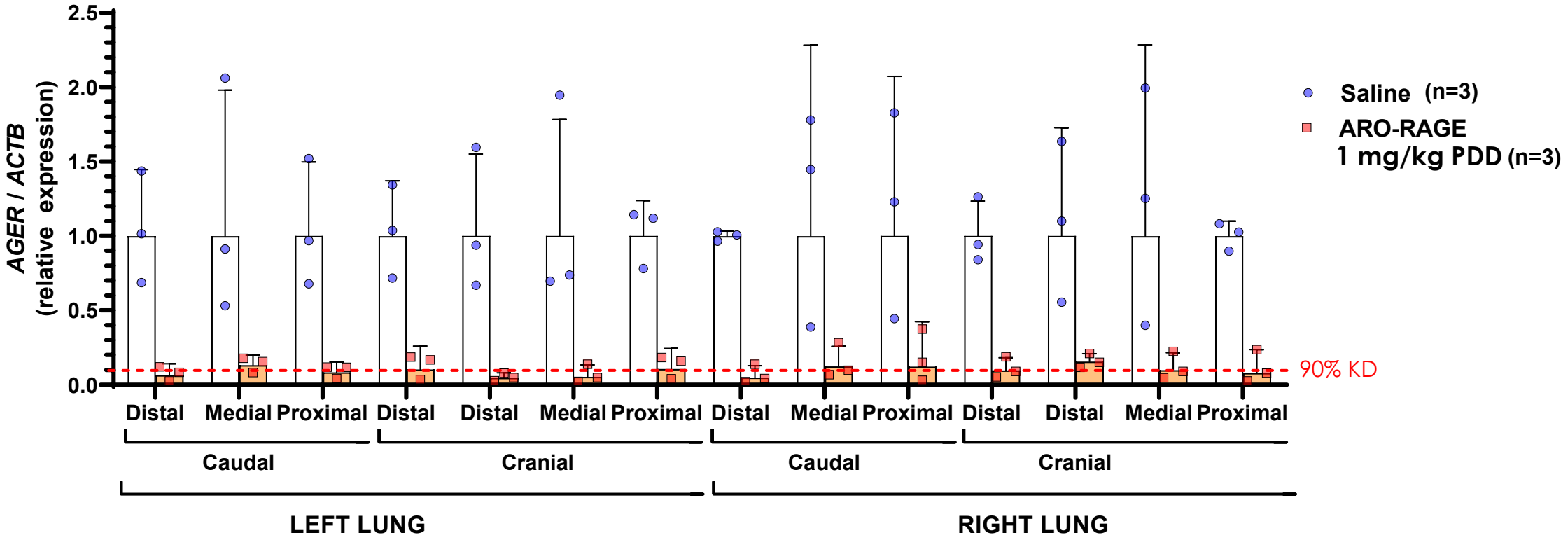
Single Inhaled Dose of ARO-RAGE Silenced >90% of Lung RAGE mRNA at Day 15 in Cynomolgus Monkeys



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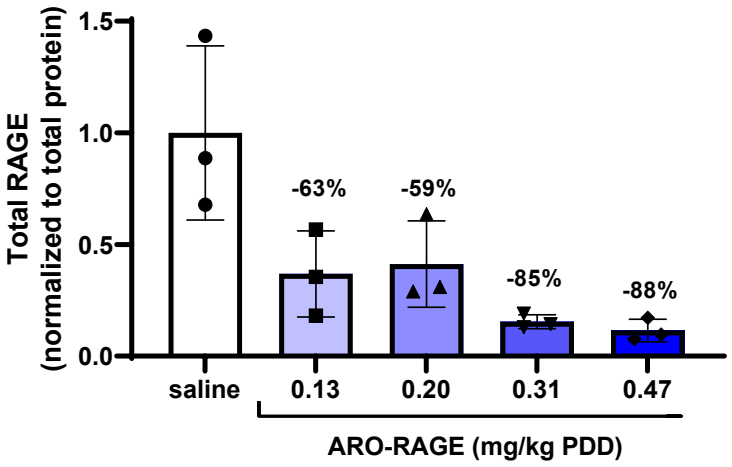
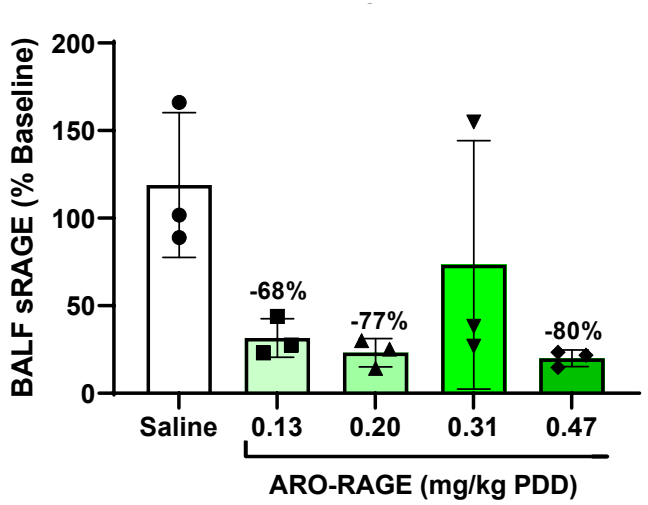
ACTB = beta actin mRNA
AGER = RAGE mRNA
PDD = pulmonary deposited dose

Single Inhaled Dose of ARO-RAGE Reduced RAGE Protein in the Lung, Airway, and Serum at Day 29 in Cynomolgus Monkeys

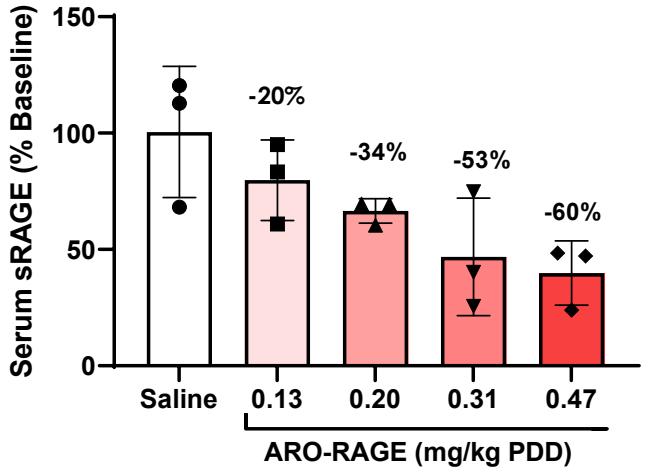
Lung Tissue RAGE Protein Day 29



BALF sRAGE Day 29



Serum sRAGE Day 29



ARO-RAGE resulted in larger reduction of RAGE protein in lung (RAGE and sRAGE) than in serum (sRAGE), potentially related to extrapulmonary sources of serum sRAGE

n=3 per treatment group

BALF = bronchoalveolar lavage fluid
PDD = pulmonary deposited dose
sRAGE = soluble RAGE

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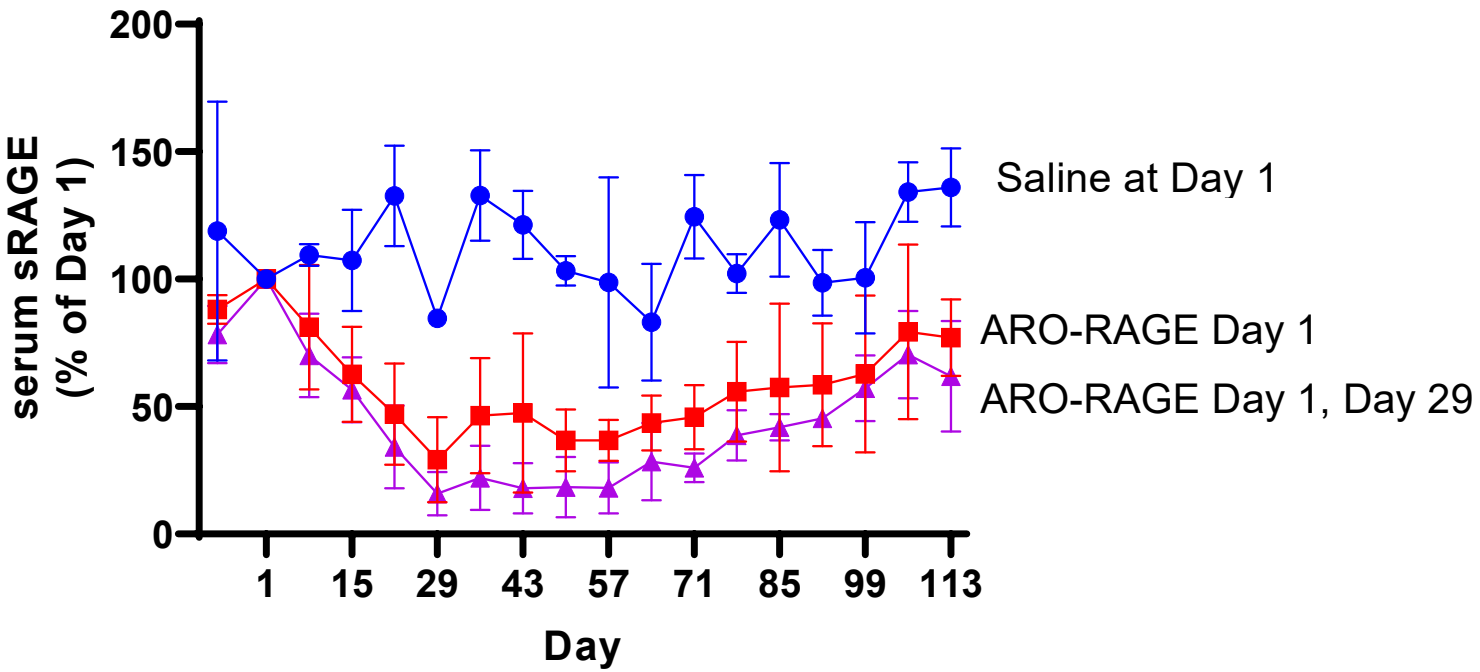
ARO-RAGE Induced Deep, Durable Reduction of Serum sRAGE in Cynomolgus Monkeys



- Single ARO-RAGE dose (1.2 mg/kg PDD) reduced serum sRAGE >50% up to 71 days
- Second ARO-RAGE dose (1.2 mg/kg PDD) on Day 29 further reduced serum sRAGE >80% at nadir
- At least 50% reduction in serum sRAGE through 92 days after second ARO-RAGE dose

n=3 per treatment group

PDD = pulmonary deposited dose
sRAGE = soluble RAGE

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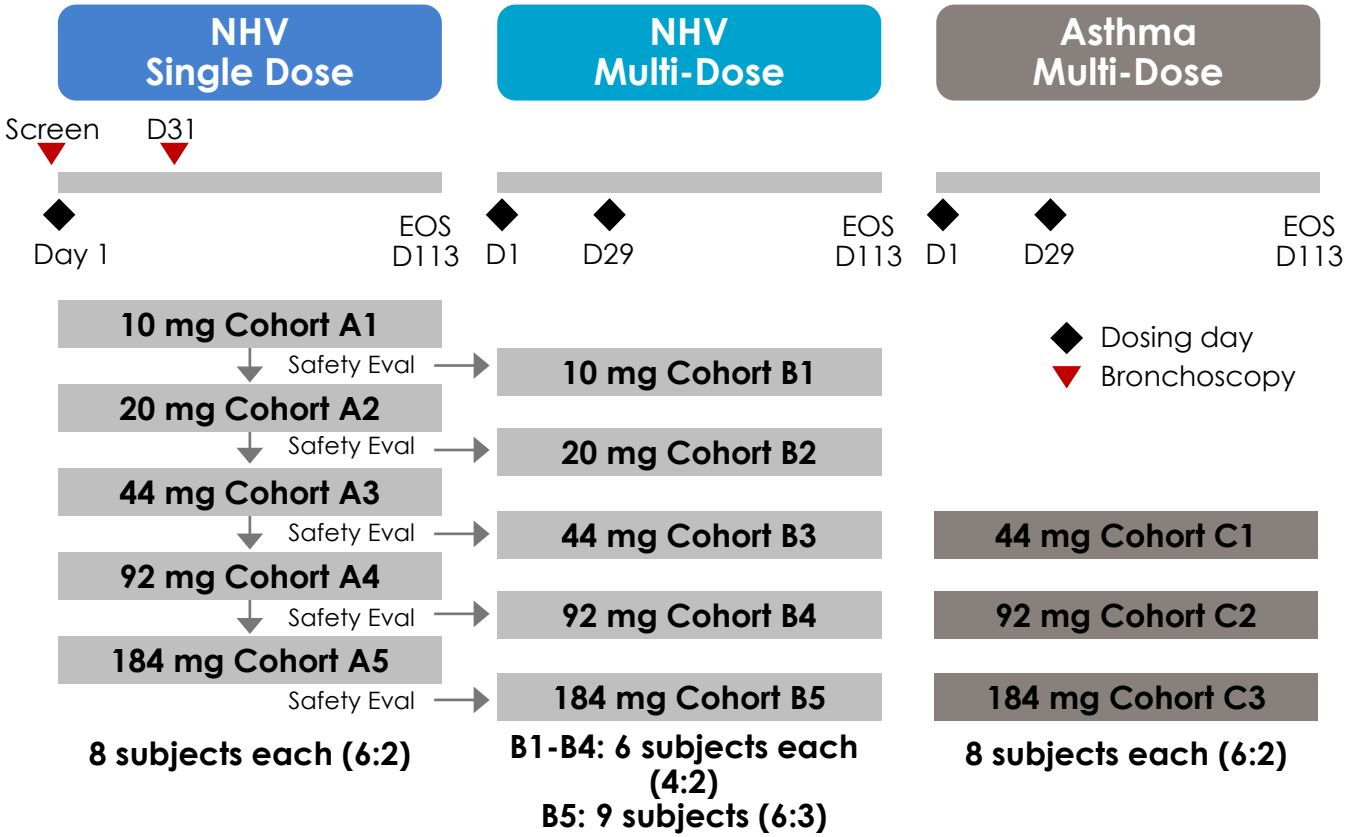
ARO-RAGE First-in-Human Study Design

- Study Design: ARORAGE-1001 (NCT05276570) is an ongoing, randomized, double-blind, placebo-controlled, phase 1/2a study of ARO-RAGE in healthy volunteers and subjects with asthma.
- Subjects:
  - Healthy volunteer cohorts: Age 18-55 years; no underlying lung disease; $ppFEV_1$ >80%; non-smoker
  - Asthma cohorts: Age 18-60 years; mild-moderate asthma (GINA 1-4); $ppFEV_1$ ≥70%; BEC ≥200 cells/ml; non-smoker
- Exposures:
  - ARO-RAGE: ascending dose levels given on Day 1 (SAD) or Days 1 and 29 (MAD)
  - Placebo: normal saline
- Endpoints:
  - Primary: TEAE incidence
  - Target engagement (exploratory):
    - Serum sRAGE
    - BALF sRAGE



BEC = blood eosinophil count
EOS = end of study
MAD = multiple ascending dose
NHV = normal healthy volunteer

$ppFEV_1$ = percent-predicted forced expiratory volume in 1 second
SAD = single ascending dose
sRAGE = soluble RAGE
TEAE = treatment-emergent adverse event

ARORAGE-1001 Key Endpoints

Safety (Pulmonary):

- Respiratory Adverse Events
- Lung function
- Inflammatory Cells: BALF Cell Count and Differential
- Chest X-rays

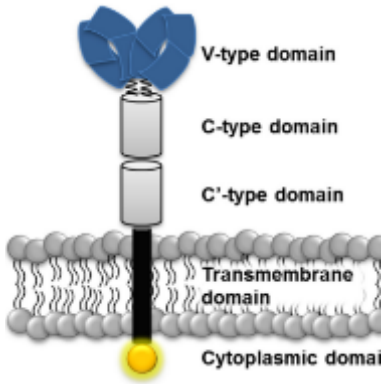
Target Engagement:

Soluble RAGE protein (sRAGE)

- Serum
- BALF

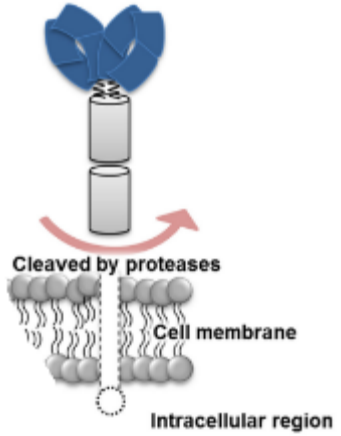
Full-length Membrane-associated RAGE (mRAGE)

Therapeutic target



Soluble RAGE (sRAGE)

Target engagement marker



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Fishman, et al, *J. Mol. Med*. 2018, 24:59

BALF = bronchoalveolar lavage fluid

ARORAGE-1001 Baseline Characteristics

Characteristic*	Healthy Volunteer (N=73)^	Asthma (N=9)^
Age – yr	34.7 ± 9.5	37.7 ± 11.9
Male – no. (%)	21 (28.8)	3 (33.3)
White – no. (%)	41 (56.2)	8 (88.9)
BMI – kg/m^2	25.7 ± 3.6	26.0 ± 3.9
Prebronchodilator $ppFEV_1$ - %	96.3 ± 10.7	93.4 ± 10.1
ICS Dose – no. (%)		
None or Undetermined	---	3 (33.3)
Low	---	5 (55.6)
Medium	---	1 (11.1)
High	---	0 (0)
Blood eosinophil – cells/μl	---	256 ± 113
FeNO – ppb	---	37.8 ± 28.9
Serum total IgE – IU/ml	---	350 ± 257
Serum sRAGE – pg/ml	1167 ± 533	1280 ± 430
BALF sRAGE – pg/ml	2487 ± 1716	---

* mean ± SD.
^ N represents entire population (ARO-RAGE + placebo) randomized to date; ongoing study remains blinded.

BALF = bronchoalveolar lavage fluid
BMI = body-mass index
FeNO = fractional exhaled nitric oxide
ICS = inhaled corticosteroid
$ppFEV_1$ = percent-predicted forced expiratory volume in 1 second
sRAGE = soluble RAGE

Data cut 17 July 2023

Interim Blinded Safety Results: Summary of Treatment-Emergent Adverse Events

Event	Healthy Volunteer SAD Cohorts (N=40)* n (%)	Healthy Volunteer MAD Cohorts (N=33)* n (%)	Asthma MAD Cohorts (N=9)* n (%)
≥1 TEAE	29 (72.5)	20 (60.6)	8 (88.9)
≥1 Serious TEAE	0 (0)	0 (0)	0 (0)
≥1 TEAE leading to trial withdrawal or study drug discontinuation	0 (0)	0 (0)	0 (0)
Most common TEAEs			
Headache	10 (25.0)	4 (12.1)	3 (33.3)
URTI	6 (15.0)	5 (15.2)	2 (22.2)
COVID-19	5 (12.5)	6 (18.2)	0 (0)
Oropharyngeal pain	3 (7.5)	6 (18.2)	0 (0)

*N represents entire population (ie ARO-RAGE + placebo) randomized to date, as the ongoing study remains blinded.
N = number of subjects in population; n = number of subjects reporting event; % = 100 x n/N.

MAD = multiple ascending dose
SAD = single ascending dose
TEAE = treatment-emergent adverse event
URTI = upper respiratory tract infection

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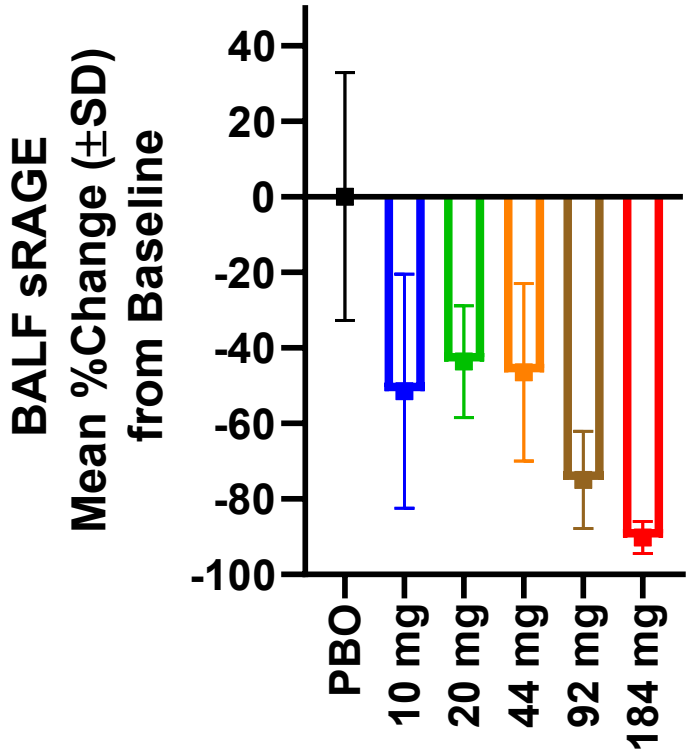
Single Dose of ARO-RAGE Resulted in Dose-Dependent Decreases in BALF sRAGE at 1 Month

Healthy Volunteer SAD Cohorts
Change from Baseline at Day 31



Treatment	BALF sRAGE % Change from Baseline*
Placebo	0.1 ± 32.8
ARO-RAGE 10 mg	-51.5 ± 31.0
ARO-RAGE 20 mg	-43.6 ± 14.8
ARO-RAGE 44 mg	-46.4 ± 23.5
ARO-RAGE 92 mg	-74.9 ± 12.9
ARO-RAGE 184 mg	-90.2 ± 4.2

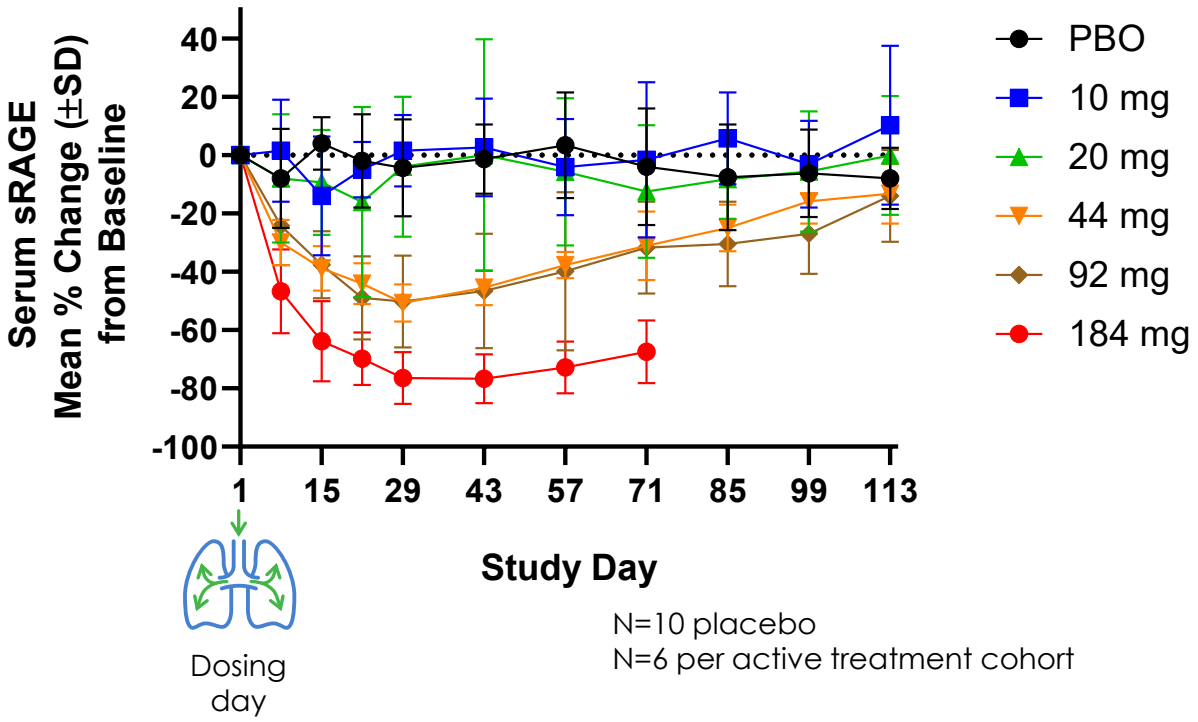
*mean ± SD.
N=10 placebo
N=6 per active treatment cohort

BALF = bronchoalveolar lavage fluid
PBO = placebo
SAD = single ascending dose
sRAGE = soluble RAGE

Data cut 18 July 2023

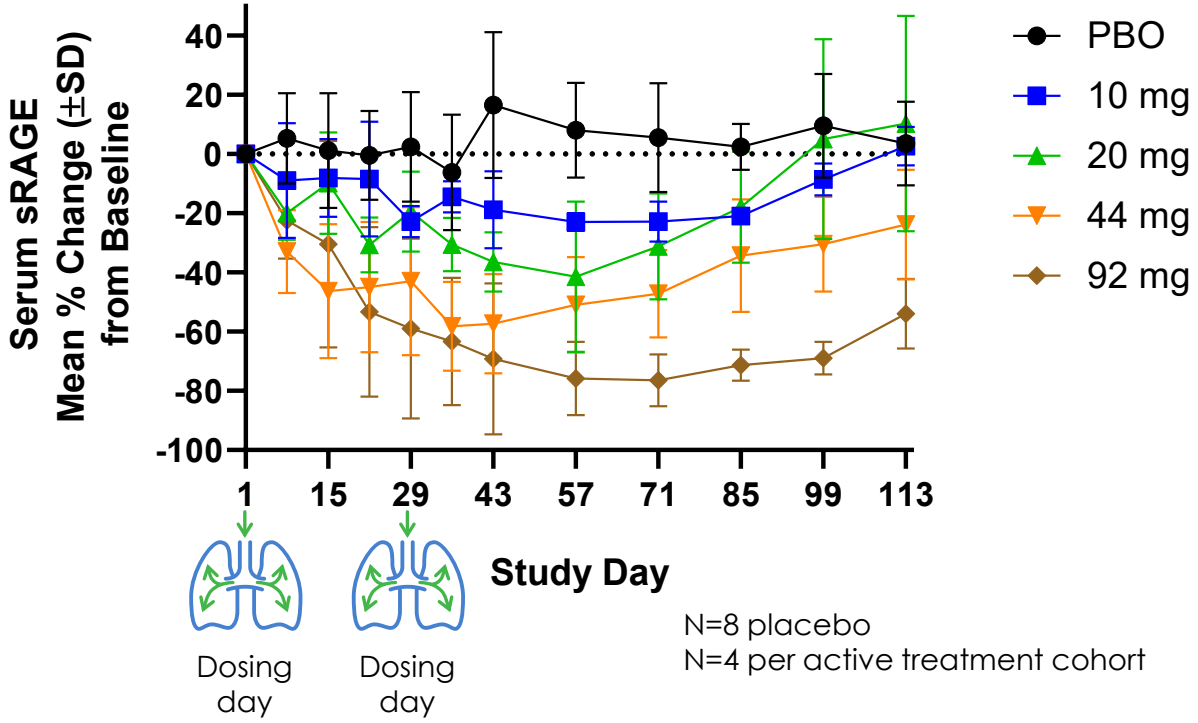
ARO-RAGE Resulted in Mean Maximum Serum sRAGE Reduction Up to 79% with Single Dose, Up to 80% with Multiple Doses

Healthy Volunteer SAD Cohorts



N=10 placebo
N=6 per active treatment cohort

Healthy Volunteer MAD Cohorts



N=8 placebo
N=4 per active treatment cohort

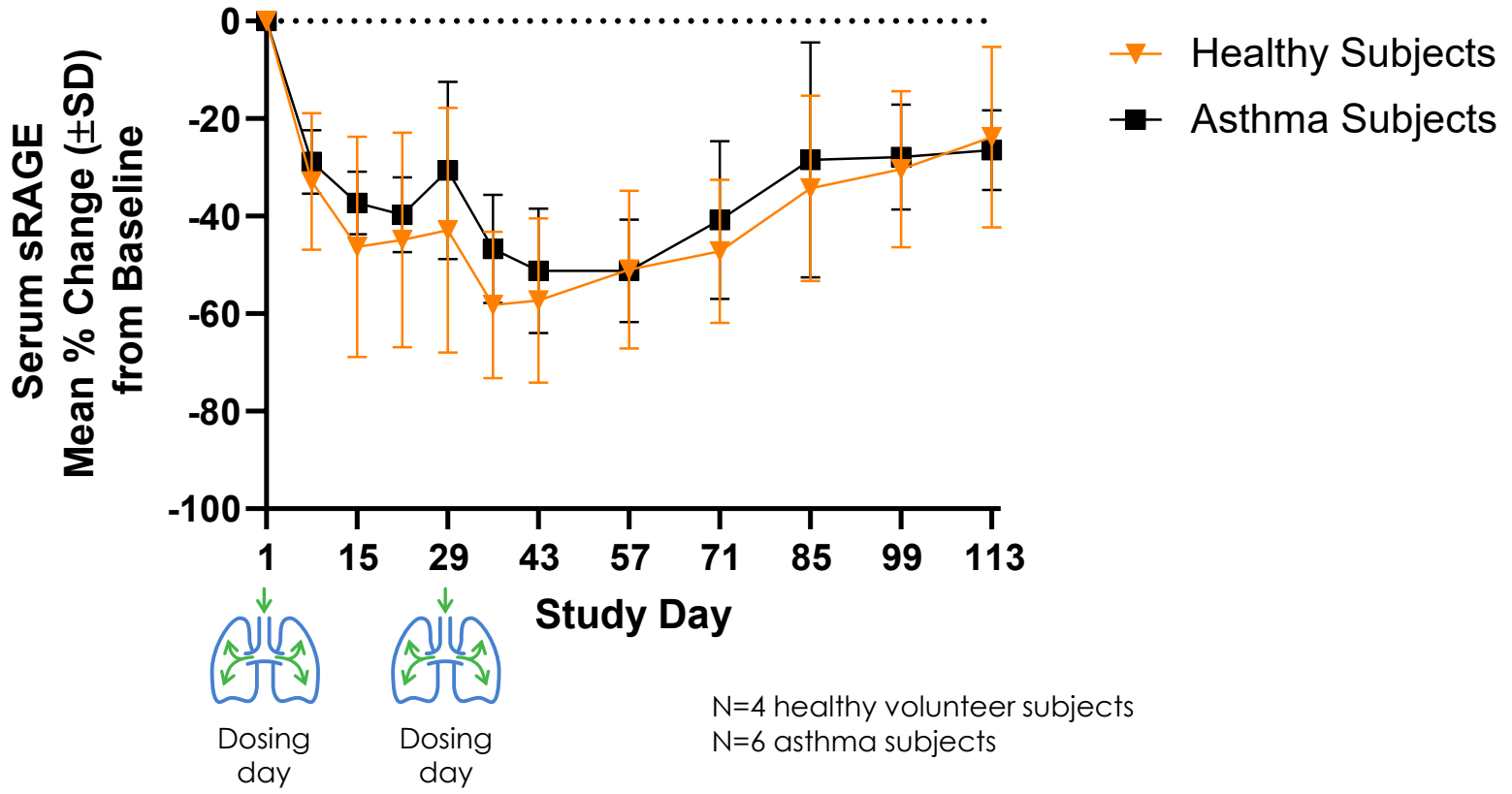
184 mg Dose Data Pending

PBO = placebo
SAD = single ascending dose
MAD = multiple ascending dose
sRAGE = soluble RAGE

Data cut 18 July 2023

ARO-RAGE Resulted in Comparable Serum sRAGE Reductions in Asthma and Healthy Subjects

44 mg Multiple Dose Cohorts: Healthy vs. Asthma



N=4 healthy volunteer subjects
N=6 asthma subjects

Data cut 21 July 2023

Conclusions

- RAGE-directed RNAi decreased RAGE expression and pulmonary inflammation in animal models.
- ARO-RAGE has been well-tolerated to date in healthy volunteers and asthma patients.
- ARO-RAGE reduced sRAGE concentration in BALF and serum in a dose-dependent manner.
- Reduction of serum sRAGE by ARO-RAGE was similar in healthy volunteers and asthma patients at the 44 mg dose level. Asthma patient enrollment is ongoing at higher doses.

Disclosures/Acknowledgments

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Co-author conflicts of interest:

J Huetsch, J Hamilton, L Moser, S Alagarsamy, D Kasahara, and E Bush are employees of and shareholders of Arrowhead Pharmaceuticals

M O'Carroll and M Salathe have received consulting fees from Arrowhead Pharmaceuticals

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