

Silencing MUC5AC Expression with a Lung-Targeted RNAi Trigger Prevents Allergen Induced Muco-obstruction and Airway Hyperresponsiveness

David I. Kasahara¹, Anthony Nicholas¹, Long Nguyen¹, Zach Triling¹, Maria Blanca-Afrazi¹, Holly Hamilton¹, Julia Hegge¹, Burton F. Dickey², Juan R. Sabater³, James Hamilton¹, Tao Pei¹, Erik W. Bush¹

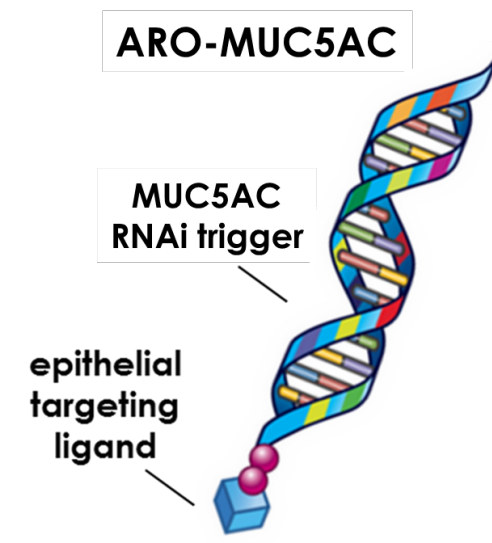
1. Arrowhead Pharmaceuticals, WI; 2. Univ. Texas M.D. Anderson Cancer Center, TX ; 3. Mount Sinai Medical Center, FL

RATIONALE

- MUC5AC is an inducible gel-forming mucin expressed by secretory airway epithelial cells in the lung
- MUC5AC expression and secretion is robustly increased in response to allergens, infection and other pro-inflammatory stimuli
- Increased MUC5AC expression in asthma, COPD and other muco-obstructive diseases contributes to small airway obstruction, reduced mucociliary clearance and functional impairment
- In mice, Muc5ac is required for allergen-induced airway hyperresponsiveness (Evans et al. 2015)
- Current asthma therapies address inflammation and bronchoconstriction, but new approaches to directly treat mucus hypersecretion and obstruction are needed
- Therapeutic small interfering RNAs (siRNAs) offer an important novel approach to selectively silence previously “undruggable” disease genes like MUC5AC

TRiM™ platform

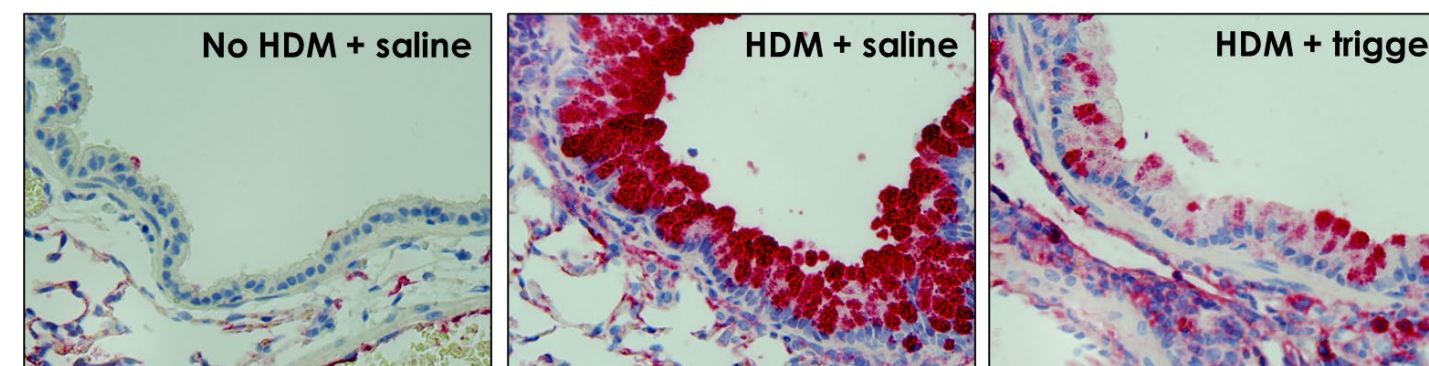
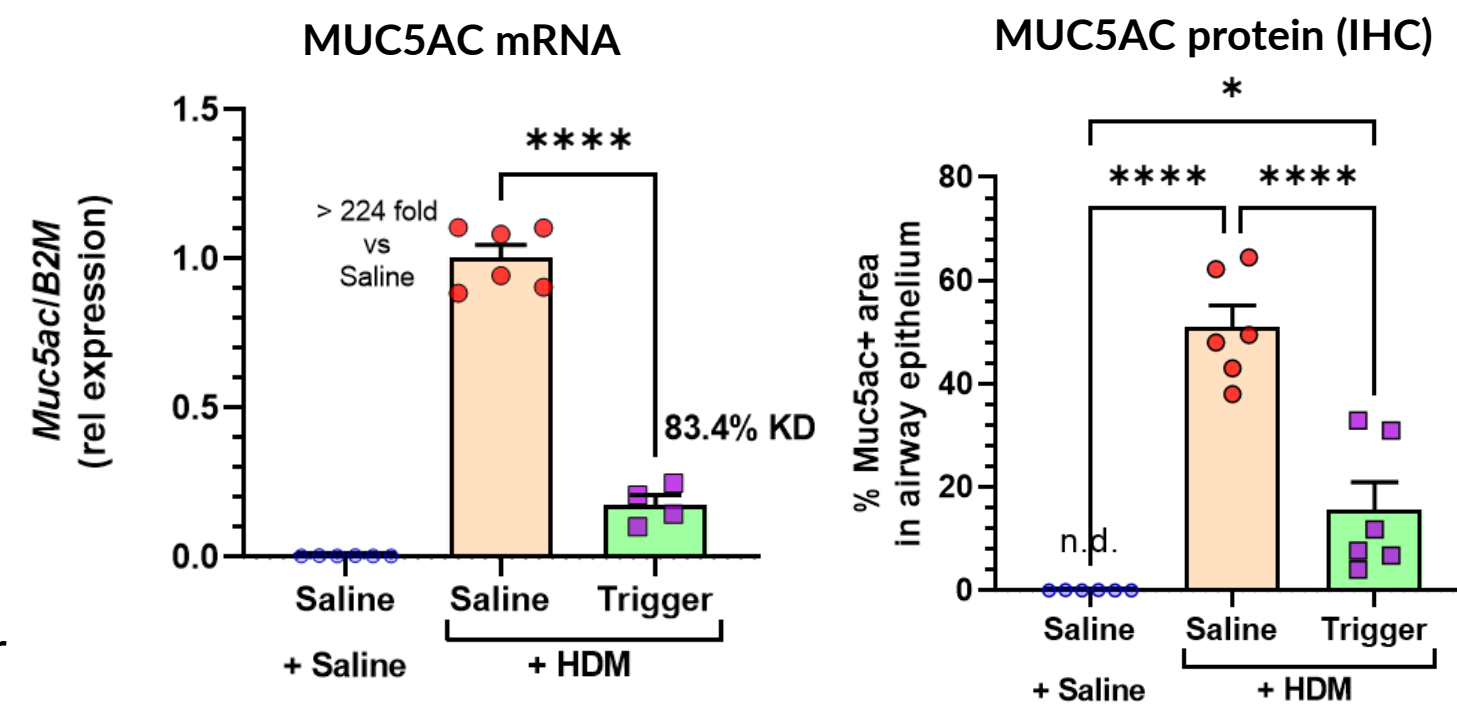
- Optimized RNAi trigger sequence for specific target gene silencing and limited potential off-target interactions
- Rational use of modifying chemistries maximize innate stability and potency
- Epithelial integrin targeting moiety facilitates trigger endocytosis



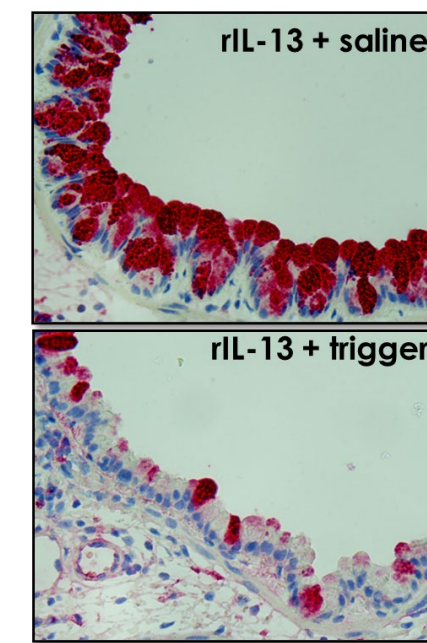
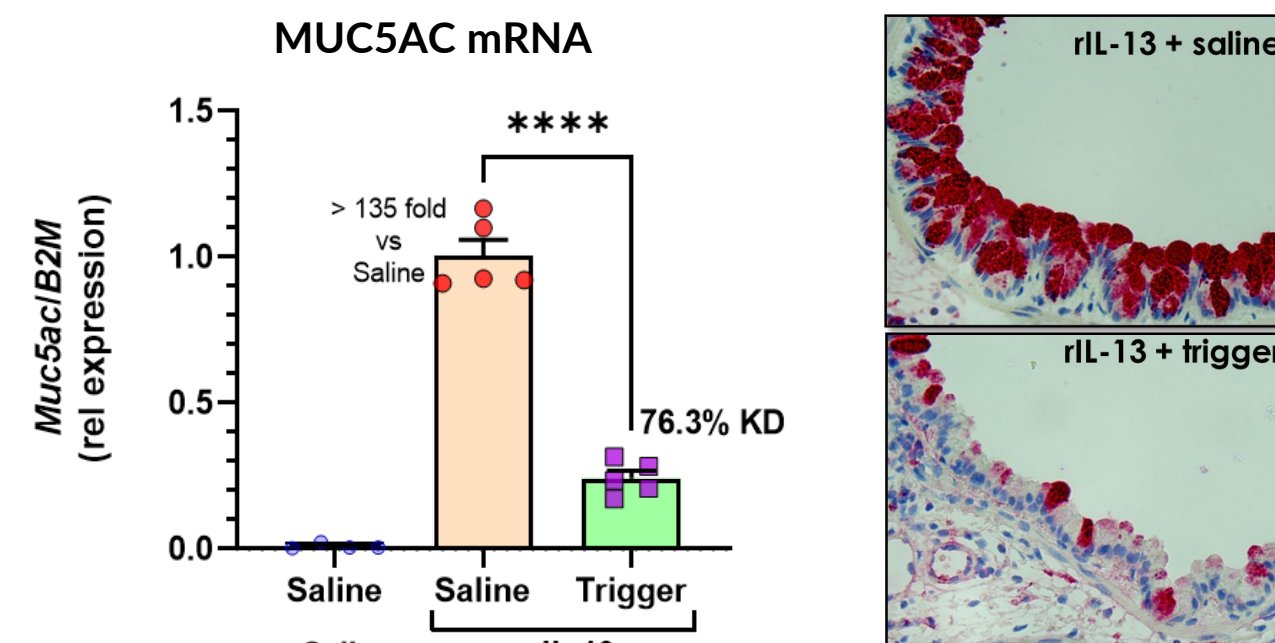
RESULTS

Muc5ac mRNA and protein silencing in mouse models of airway inflammation

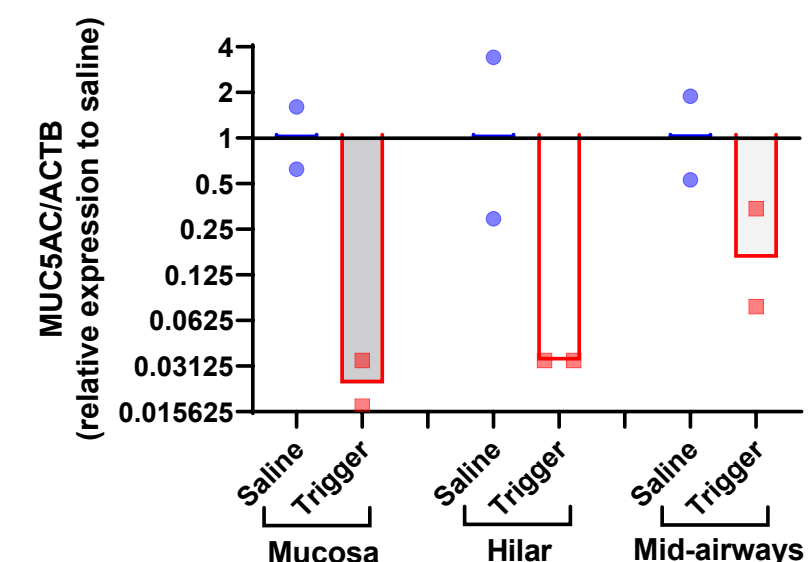
House dust mite (HDM) allergen induced a >200-fold increase in lung Muc5ac mRNA expression which was significantly reduced by treatment with a Muc5ac-targeting RNAi trigger conjugate (upper left panel). A similar pattern was observed for airway epithelial Muc5ac protein in lung tissue sections stained by IHC (middle panels) and quantitated by HALO image analysis (upper right panel).



In a second model of airway inflammation, Muc5ac trigger-treated mice were challenged with i.n. rIL-13. Robust IL-13-mediated increases in lung Muc5ac mRNA (lower left panel) and airway Muc5ac protein (lower right panel) were significantly attenuated in treated animals.



MUC5AC silencing in non-human primates



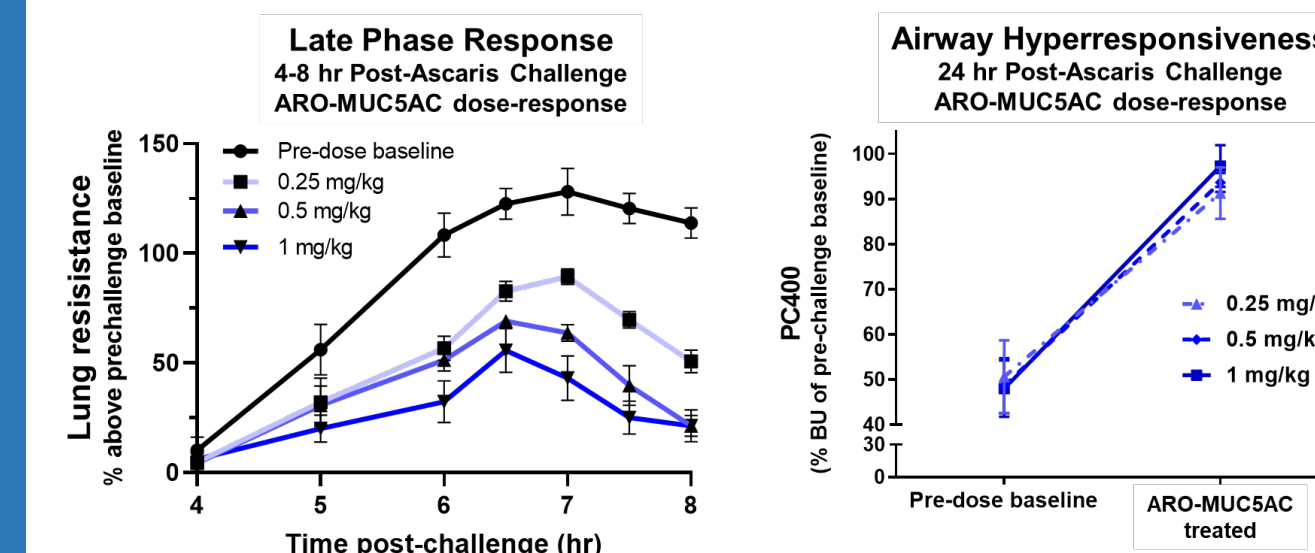
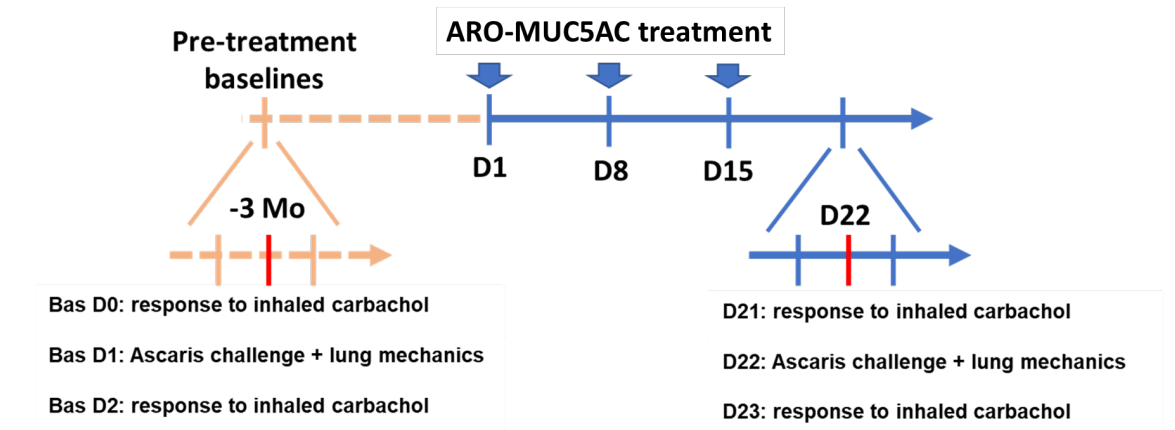
Expression level of MUC5AC mRNA normalized to saline control (geometric mean of expression=1)

- Intubated cynomolgus monkeys received aerosolized MUC5AC-targeting RNAi trigger conjugate (1 mg/kg, n=2) or saline (n=2) on days 1, 8 and 15.
- On day 22, lung (right cranial lobe) tissue samples were collected from airway mucosa, hilar region or mid-airway.
- Inhaled trigger treatment reduced MUC5AC mRNA expression in all three regions, with the deepest silencing (>90%) observed in airway mucosa.

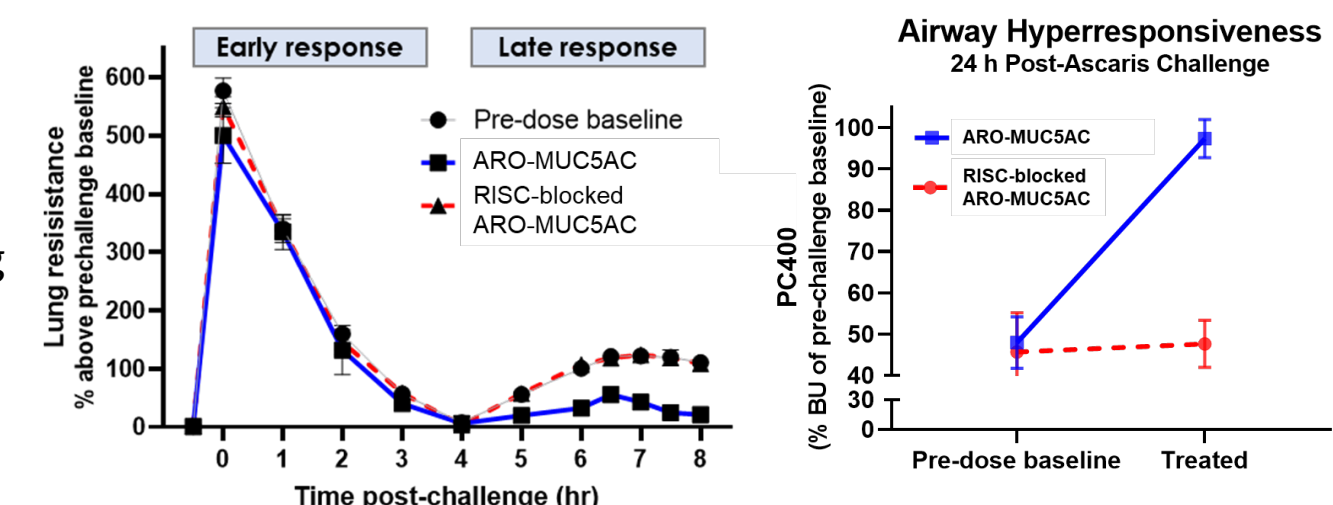
ARO-MUC5AC attenuates late-phase reaction and airway hyperresponsiveness in a sheep model of allergic asthma

After baseline pre-dose airway responses to Ascaris challenge were measured in sensitized sheep, animals received three weekly (days 1, 8, 15) inhaled doses of ARO-MUC5AC. Three dose levels of ARO-MUC5AC were evaluated: 0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg pulmonary deposited dose (PDD).

A week after the third dose, airway responses to Ascaris challenge were evaluated. Late-phase airway response (~125% increase in lung resistance 7 hours post-challenge) was dose-dependently attenuated by ARO-MUC5AC treatment (below, left). At 24 hours post-challenge, carbachol-induced airway hyperresponsiveness was robustly suppressed at all dose levels of ARO-MUC5AC (below, right).



In contrast to ARO-MUC5AC (1 mg/kg), RISC-blocked ARO-MUC5AC (1 mg/kg) treatment had no effect on late phase response to allergen challenge (left panel) or airway hyperresponsiveness to carbachol (right panel). These results highlight a critical role for MUC5AC in mediating pathological airway responses to allergen challenge in sensitized sheep, particularly during the late phase response and in combination with bronchoconstriction.



Allergen challenge was repeated on day 51 (5 weeks after the last dose of ARO-MUC5AC) and airway responses had fully returned to pre-dose baseline parameters (data not shown). As a negative control, sheep were dosed with a version of ARO-MUC5AC modified to block RISC incorporation and prevent MUC5AC mRNA silencing (1 mg/kg PDD on days 1, 8, 15; day 22 challenge and evaluation).

CONCLUSIONS

- Inhaled epithelial-targeted RNAi trigger conjugates effectively silence allergen- and IL-13-induced MUC5AC expression and confirm the pathogenic role of this inducible mucin in late phase allergic response and airway hyperresponsiveness.
- ARO-MUC5AC represents the first siRNA to silence mucus expression in the lung. Arrowhead plans to initiate a Phase 1/2a clinical trial to evaluate ARO-MUC5AC as a potential treatment for asthma and other muco-obstructive lung diseases.