Silencing TSLP expression with a lung-targeted RNAi molecule suppresses pulmonary allergic inflammation

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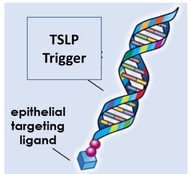
INTRODUCTION

- Thymic stromal lymphopoietin (TSLP), an epithelial cell-derived cytokine, promotes airway inflammation by activating Th2 cells, ILC2, and myeloid dendritic cells (mDCs) via the TSLP receptor (TSLPR).
- TSLP is increased in the serum, BALF, induced sputum, and airway epithelium of asthma patients and is correlated with the severity of asthma.
- Selective silencing of *TSLP* mRNA expression via delivery of lung-targeted therapeutic small interfering RNA (siRNA) limits pulmonary inflammation in animal models and is a promising approach for the treatment of asthma.

AIMS

- We utilized Arrowhead's pulmonary epithelial Targeted RNAi Molecule (TRiM™) delivery platform to:
- Optimize TSLP siRNAs (RNAi triggers) that potently and durably silence TSLP mRNA and protein expression in rat and AAV-transduced mouse lung.
- Demonstrate that silencing lung TSLP expression effectively suppresses pulmonary inflammation in a rat model of allergic asthma.



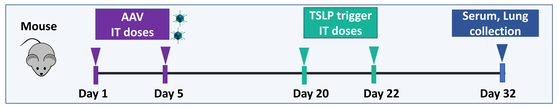
METHODS

Rodent studies:

- Rats received two intratracheal (IT) doses of a rat-specific RNAi trigger targeting *TSLP* mRNA 2 weeks before Alternaria (Alt) challenge.
- BAL and lung tissues were collected 2-24 hours post-challenge for quantitation of *TSLP* expression and evaluation of inflammatory markers.

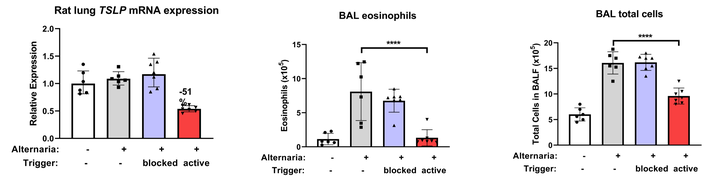
AAV9-CAG-hTSLP transduced mouse model study:

- AAV9-CAG-hTSLP vector was co-dosed with AAV9-CAG-eGFP in C57Bl/6 mice intratracheally on Study Days 1 and 5 followed by vehicle (saline) or siRNA administration on Study Days 20 and 22.
- Serum and lung samples were collected on Study Day 32 for qPCR (normalized to eGFP) and hTSLP V-Plex MSD assay.



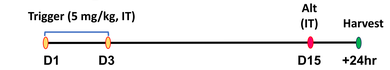
RESULTS

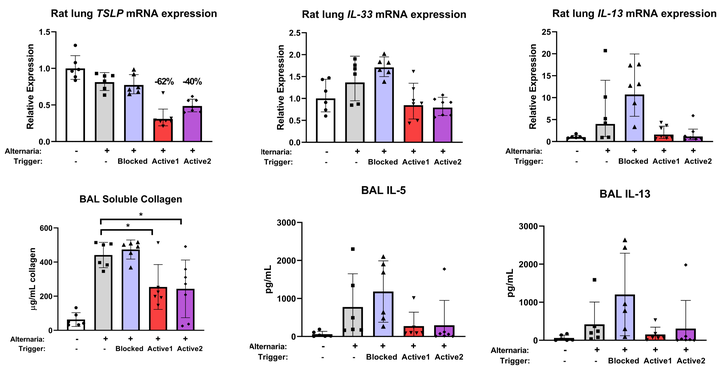
Silencing *TSLP* reduces BAL inflammatory cells in rat allergic asthma model



- Sprague-Dawley rats were treated intratracheally (IT) with either saline or 5 mg/kg of rat-specific TSLP trigger once daily on Days 1 and 3. A non-RISC loading version of the trigger was used as a negative control. On Day 13, rats were subjected to intratracheal challenge with vehicle (saline) or 500 µg of Alternaria alternata extract. Lung tissues and BAL samples were collected 2 hours after Alternaria challenge.
- Relative to vehicle controls, whole lung *TSLP* mRNA expression was reduced by 51% in TSLP trigger-treated rats.
- BAL eosinophils and total cells were increased 2 hours post Alternaria challenge. TSLP trigger treatment significantly limited Alternaria-induced increases in BAL eosinophils and total cells; the non-RISC loading version control trigger had no effect on lung *TSLP* expression or Alternaria-induced BAL inflammatory cell counts.
- Data are analyzed by one-way ANOVA.(MEAN with SD; *P<0.05, **P<0.01)

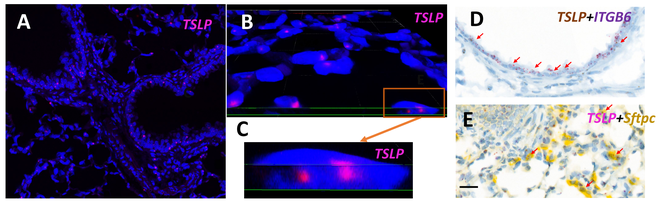
Silencing *TSLP* reduces Th2 cytokines in rat allergic asthma model





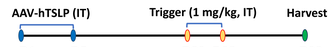
- Sprague-Dawley rats were treated intratracheally with either saline or 5 mg/kg of rat-specific TSLP trigger once daily on Days 1 and 3. On Day 15, rats were subjected to intratracheal challenge with vehicle (saline) or 500 µg of Alternaria alternata extract. Lung tissues and BAL samples were collected 24 hours after Alternaria challenge.
- Relative to vehicle controls, triggers targeting two different mRNA positions reduced whole lung *TSLP* mRNA expression by 62% and 40%, respectively. Th2 cytokines *IL13* and *IL33* mRNA are decreased treated with both active triggers relative to vehicle control or negative trigger.
- Soluble collagen, Th2 cytokines IL13 and IL33 in BAL were increased 24 hours post Alternaria challenge. In contrast, all of them were reduced in the BAL treated with TSLP trigger relative to vehicle control or negative trigger.
- Data are analyzed by one-way ANOVA.(MEAN with SD; *P<0.05, **P<0.01)

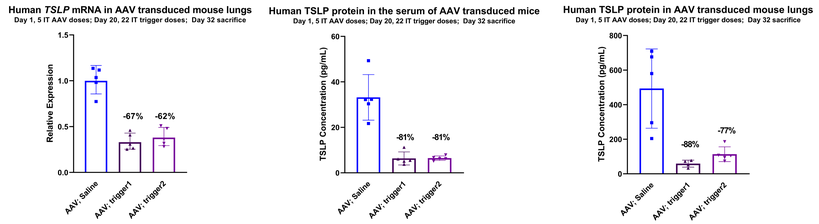
TSLP is expressed in airway and alveolar epithelium; silencing of cytoplasmic *TSLP* mRNA does not reduce pre-mRNA retained in nucleus



- 7-week-old Sprague-Dawley rat lungs were inflated, fixed in 4% PFA and processed for RNAscope mRNA in situ hybridization and immunohistochemistry (IHC).
- *TSLP* RNAscope shows TSLP is expressed in airway and alveolar (Fig. A). Z-stack confocal scan images show *TSLP* transcript retained in the nucleus (Fig. B, C). Duplex RNAscope of TSLP and ITGB6 confirmed expression in airway epithelium (Fig. D).
- Co-staining of TSLP RNAscope and Sftpc IHC demonstrated TSLP expression in alveolar type 2 cells (Fig. E). Scale bar=20 µM.

Candidate therapeutic siRNAs targeting human *TSLP* significantly reduce human mRNA and protein expression in AAV-transduced mouse lungs and serum





- Mice were dosed intratracheally (IT) with AAV-hTSLP to induce stable human *TSLP* mRNA expression in the lung. Saline or siRNA triggers were dosed after human TSLP expression stabilized on Day 20 and Day 22. Serum and lung samples were collected on Day 32 for qPCR and MSD assay.
- Relative to vehicle controls, triggers targeting two different mRNA positions reduced whole lung human *TSLP* mRNA expression by 67% and 62%, respectively.
- Corresponding reductions in human TSLP protein (~80%) in lung and serum were more substantial than mRNA and may reflect an unsilenced (but untranslated) nuclear pre-mRNA pool.

CONCLUSIONS

- **Lung epithelium-targeted siRNAs effectively silence *TSLP* expression and limit pulmonary inflammation in a rat model of allergic asthma.**
- **Human cross-reactive candidate therapeutic siRNAs silence human TSLP mRNA and protein in an AAV-hTSLP mouse screening model.**
- **Selectively targeting lung TSLP with inhaled therapeutic siRNA offers a novel approach for the potential treatment of allergic asthma.**