Lung-targeted RNAi molecules silence TSLP expression in human PCLS cultures and humanized mice, and suppress pulmonary allergic inflammation

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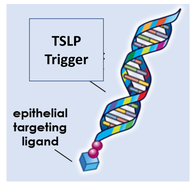
INTRODUCTION

- The epithelial cytokine thymic stromal lymphopoietin (TSLP) is a genetically and clinically validated therapeutic target that activates multiple immune cell lineages to promote asthmatic inflammation.
- ARO-TSLP is an epithelial integrin-targeted siRNA conjugate that specifically silences human TSLP mRNA.
- Inhaled ARO-TSLP may offer a novel approach for the treatment of asthma.

AIMS

We utilized Arrowhead's pulmonary epithelial Targeted RNAi Molecule (TRiM™) delivery platform to:

- Demonstrate that silencing lung TSLP expression effectively suppresses pulmonary inflammation in a rat model of allergic asthma.
- Optimize TSLP siRNAs (RNAi triggers) that potently and durably silence TSLP mRNA and protein expression in cultured human precision cut lung slices (hPCLSs), humanized hTSLP/hTSLPR double knock-in mice, and AAV9-CAG-hTSLP transduced mice.



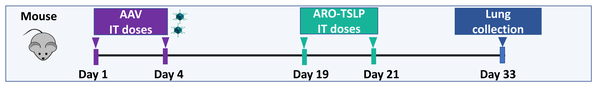
METHODS

Rat *Alternaria* model of allergic asthma:

- Rats received two intratracheal (IT) doses of a rat-specific RNAi trigger targeting *TSLP* mRNA 2 weeks before *Alternaria* allergen (Alt, 500 µg) challenge. Lung tissues and bronchoalveolar lavage (BAL) samples were collected 2 hours after *Alternaria* challenge.

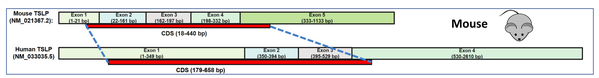
AAV9-CAG-hTSLP transduced mouse model study:

- AAV9-CAG-hTSLP was co-dosed with AAV9-CAG-eGFP in C57Bl/6 mice IT on Study Days 1 and 4 followed by vehicle (saline) or ARO-TSLP on Study Days 19 and 21. Lung tissues were collected on Day 33 for qPCR (normalized to eGFP) and hTSLP V-Plex MSD assay.



Humanized TSLP mouse model:

- In humanized TSLP/TSLPR mice, the mouse TSLP transcript is replaced by the human TSLP transcript. Humanized mice received IT doses of saline or ARO-TSLP on Day 1 and Day 3. Lung tissues were collected on Day 15 for qPCR analysis.

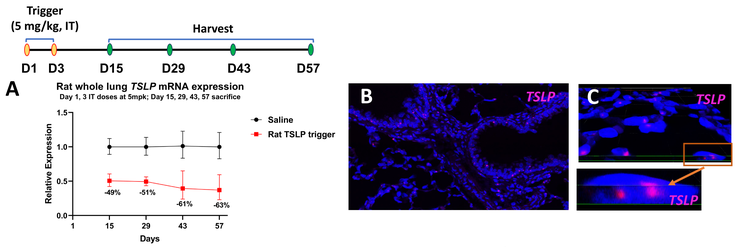


Human precision cut lung slices (hPCLSs) studies:

- hPCLSs from healthy or asthmatic donors (ReproCELL Europe Ltd) were exposed to saline or ARO-TSLP for 7 days and were collected on Day 8 for qPCR analysis.

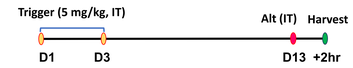
RESULTS

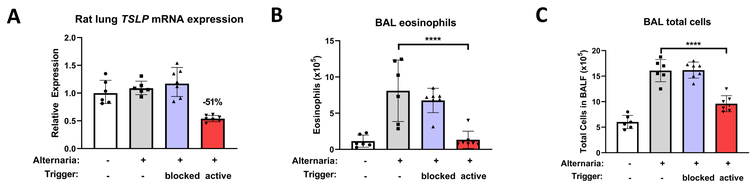
Cytoplasmic *TSLP* mRNA silencing does not reduce nuclear *TSLP* pre-mRNA



- Sprague-Dawley rats were treated intratracheally (IT) with either saline or 5 mg/kg of rat-specific TSLP trigger on Days 1 and 3. Lung tissues were collected on Days 15, 29, 43, and 57. Relative to vehicle controls, whole lung *TSLP* mRNA expression was reduced by 63% on Day 57 in TSLP trigger-treated rats (Fig. A).
- *TSLP* RNAscope shows *TSLP* mRNA is expressed in airway and alveolar epithelial cells (Fig. B). Z-stack confocal scan images show *TSLP* transcript retained in the nucleus (Fig. C), suggesting that partial *TSLP* mRNA silencing may be a consequence of nuclear pre-mRNAs that are inaccessible to the siRNA-loaded RISC complex in the cytoplasm.

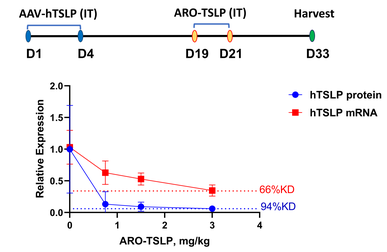
Silencing *TSLP* reduces BAL inflammatory cells in rat allergic asthma model





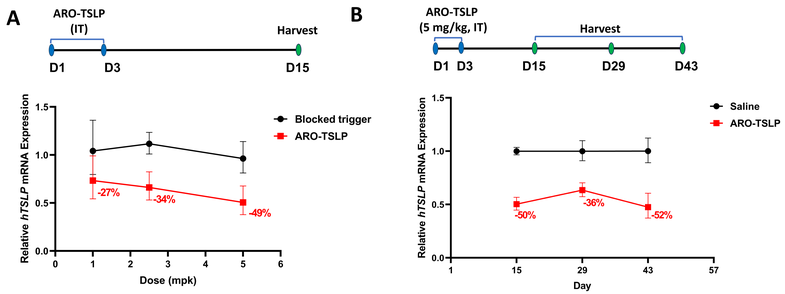
- Sprague-Dawley rats were treated intratracheally (IT) with either saline or 5 mg/kg of rat-specific TSLP trigger ("active") on Days 1 and 3. A modified version of the active trigger that is incapable of RISC loading was used as a negative control ("blocked"). On Day 13, rats were subjected to IT challenge with vehicle (saline) or *Alternaria* (Alt). Two hours later, lung tissue and BAL samples were collected.
- Relative to vehicle controls, whole lung *TSLP* mRNA expression was reduced by 51% in TSLP trigger-treated rats (Fig. A).
- BAL eosinophils and total cells were increased two hours post Alternaria challenge. TSLP trigger treatment significantly limited *Alternaria*-induced increases in BAL eosinophils (Fig. B) and total cells (Fig. C); the non-RISC loading control trigger had no effect on lung *TSLP* expression or *Alternaria*-induced BAL inflammatory cell counts.
- Data are analyzed by one-way ANOVA (mean ± SD; ****P<0.0001).

ARO-TSLP significantly reduce human TSLP mRNA and protein expression in AAV-transduced mouse lungs



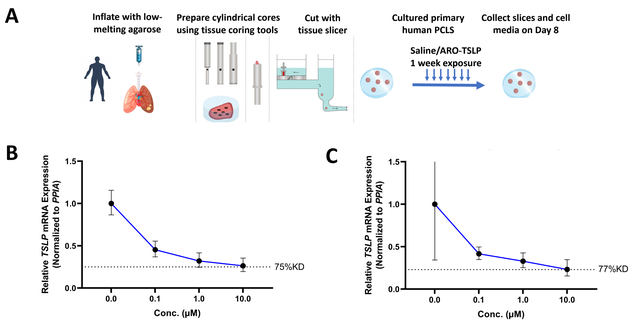
- To study human reactive clinical candidate siRNAs, mice were dosed intratracheally (IT) with AAV9-hTSLP to induce stable human *TSLP* mRNA expression in the lung. Saline or ARO-TSLP (trigger), a clinical candidate targeting human TSLP (hTSLP) transcript, were dosed on Day 19 and Day 21 after hTSLP expression stabilized. Lung samples were collected on Day 33 to measure hTSLP mRNA by qPCR and hTSLP protein by MSD assay.
- ARO-TSLP reduced whole lung hTSLP mRNA and protein expression in dose-dependent manner.
- Deeper reductions in hTSLP protein (94%) than mRNA (66%) in lung may reflect an unsilenced (but also untranslated) nuclear pre-mRNA pool.

ARO-TSLP silences human *TSLP* in humanized mouse model



- Humanized mice were treated intratracheally (IT) with either saline or ARO-TSLP, a clinical candidate targeting human *TSLP* (*hTSLP*) mRNA, at 1, 2.5, or 5 mpk on Days 1 and 3. A non-RISC loading version of the trigger was used as a negative blocked control. On Day 15, lung tissues were collected. Relative to saline group, ARO-TSLP reduced whole lung *hTSLP* mRNA expression in a dose dependent manner (49% KD at 2 x 5 mpk) (Fig. A).
- To evaluate the duration of effect, humanized mice were dosed IT with saline or ARO-TSLP (5 mpk) on Days 1 and 3, and lung tissues were harvested on Day 15, 29, or 43. *hTSLP* mRNA levels were reduced through 6 weeks following IT doses of ARO-TSLP (Fig. B).

ARO-TSLP silences human *TSLP* mRNA in healthy and asthmatic human PCLS



- Human precision-cut lung slices (PCLS) were collected from healthy or asthmatic donor and exposed to ARO-TSLP (0.1, 1 or 10 µM) for 1 week (Fig. A).
- ARO-TSLP reduced human *TSLP* mRNA in a dose-dependent manner in PCLS from healthy donors (up to 75% reduction, Fig. B) and from asthmatic donors (up to 77% reduction, Fig. C).

CONCLUSIONS

- **Lung epithelium-targeted siRNAs effectively silence *TSLP* expression and limit pulmonary inflammation in a rat model of allergic asthma.**
- **ARO-TSLP silences human TSLP in AAV9-hTSLP transduced mice, humanized TSLP mice, and primary human PCLS cultures.**
- **Selectively targeting TSLP expression in the lung with an inhaled therapeutic siRNA offers a novel potential approach for the treatment of allergic asthma.**