A clinical-stage RNAi therapeutic candidate for IPF mediates durable MMP7 silencing in non-human primates and human lung tissue

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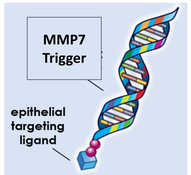
INTRODUCTION

- **In idiopathic pulmonary fibrosis, matrix metalloproteinase 7 (MMP7) overexpression promotes fibrosis and inflammation and is linked to disease severity.**
- **Previous work established that lung-targeted MMP7 siRNAs effectively limit fibrosis and improve function in a rat bleomycin injury model.**
- **Lung-selective silencing of MMP7 expression in rodent, non-human primate and human lung tissue was achieved with the use of Arrowhead's inhaled pulmonary epithelial Targeted RNAi Molecule (TRiM™) delivery platform.**

AIMS

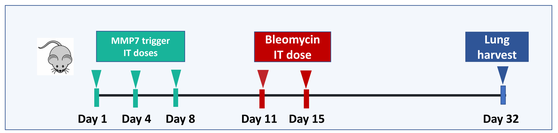
- **Determine the expression pattern of MMP7 upregulation in the bleomycin-injured rat lung and its response to silencing with lung-targeted siRNA.**
- **Evaluate the pharmacodynamic properties of an inhaled clinical-stage therapeutic siRNA (ARO-MMP7) in non-human primates and in cultured human precision cut lung slices (PCLS).**



METHODS

Rat studies

- **Prior to bleomycin (BLM) injury, rats received three intratracheal (IT) doses of saline vehicle, a rat-specific RNAi trigger targeting *MMP7* mRNA, or an inactive negative control trigger conjugate modified to prevent incorporation into the RISC complex.**
- **Three weeks later, lung tissues were processed for RNAscope in situ hybridization.**



Non-human primate study

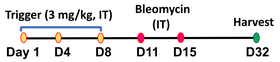
- **After baseline bronchoalveolar lavage (BAL) collections on Day -53, three male cynomolgus monkeys received a single inhaled dose of either aerosolized vehicle (sterile isotonic saline) or ARO-MMP7 via an endotracheal tube. Pulmonary deposited dose (PDD) was calculated based on minute ventilation and exposure duration.**
- **Serial post-dose BAL samples were collected on Day 15, 37, 57 and 85 for MMP7 protein quantitation via Gyros immunoassay.**

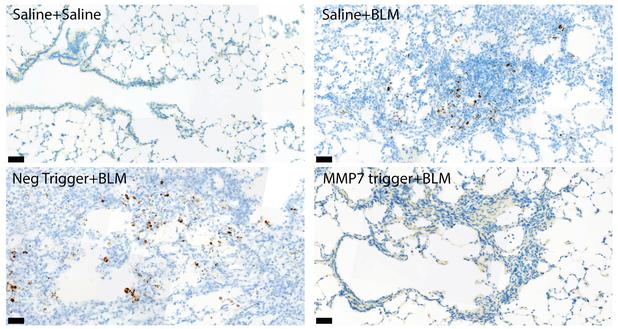
Human precision cut lung slice (PCLS) study (Reprocell)

- **Fresh lung slices from a healthy donor were cultured and exposed to vehicle (saline) or ARO-MMP7 (0.1, 1 or 10 µM) for seven days.**
- **Slices were collected on Day 8 for analysis of *MMP7* mRNA expression by qRT-PCR. Cell media on Day 8 were collected for analysis of MMP7 protein expression (MSD immunoassay) and MMP7 enzymatic activity (QuickZyme Human MMP-7 Activity Assay).**

RESULTS

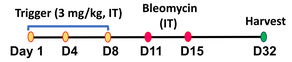
A pulmonary epithelial-targeted RNAi trigger silences *MMP7* mRNA expression in the bleomycin-injured rat lung

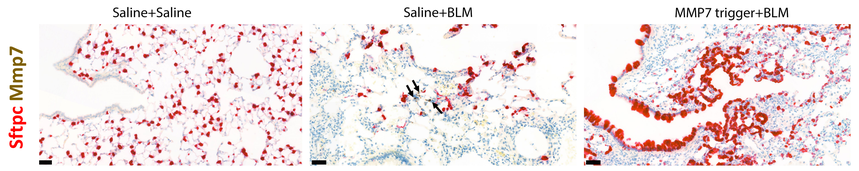




- On Days 1, 4 and 8, Sprague-Dawley rats received IT doses of either saline vehicle or 3 mg/kg of rat-specific MMP7 RNAi trigger conjugate or inactive negative control conjugate. Bleomycin (2U/kg) or saline was dosed IT on Days 11 and 15. Lung tissues were collected on Day 32 for RNAscope in situ hybridization analysis.
- Little or no *MMP7* mRNA expression is observed in uninjured regions of the lung.
- *MMP7* mRNA is highly expressed in bleomycin-injured lesions near the bronchioalveolar duct junctions invading into the lung parenchyma.
- MMP7 significantly reduces MMP7 expression in the rat bleomycin injured lung.
- Scale bar=50 µM; BLM=Bleomycin

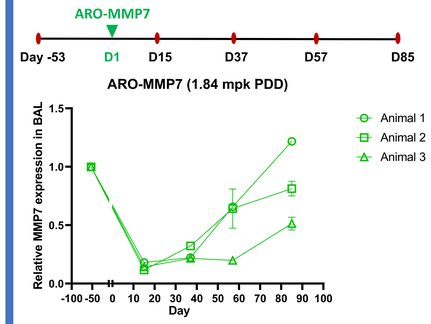
Silencing MMP7 preserves alveolar epithelium and limits injury in the rat bleomycin model





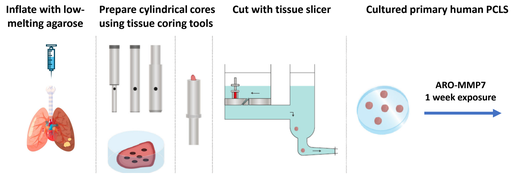
- Duplex RNAscope was performed on sections from Day 32 lung samples to visualize alveolar epithelial type 2 (AEC2) cell marker *Sftpc* mRNA (red) and *MMP7* mRNA (brown) expression *in situ*.
- Bleomycin injury resulted in significant loss of *Sftpc*+ AEC2 cells and accompanying fibrosis.
- Black arrows highlight *MMP7*+ cells in injured lesions.
- Silencing *MMP7* mRNA expression limited bleomycin injury and was associated with more *Sftpc*+ epithelial cells, consistent with enhanced alveolar regeneration.
- Scale bar=50 µM

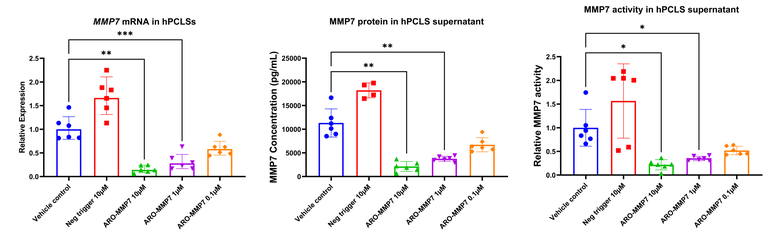
One dose of ARO-MMP7 silences >80% MMP7 protein in non-human primate BAL



- On Day 1, three cynomolgus monkeys received a single inhaled aerosol exposure of ARO-MMP7 (1.84 mg/kg pulmonary deposited dose (PDD)).
- MMP7 protein was quantitated in BAL samples collected on Day 15, 37, 57 and 85 (pre-dose baseline BAL collections were on Day -53).
- On Day 15 (two weeks post-dose), BAL MMP7 protein was reduced >80% (relative to their pre-dose baseline) in all three exposed animals.
- BAL MMP7 protein expression recovered slowly over two to three months post-dose.

ARO-MMP7 silences MMP7 mRNA and protein *ex vivo* in cultured human precision cut lung slices (hPCLS)





- Human PCLS were exposed to ARO-MMP7 (0.1, 1 or 10 µM) or inactive negative control conjugate for 1 week.
- ARO-MMP7 dose-dependently reduced human *MMP7* mRNA (max. 86% reduction at 10 mM). Data are normalized to *PPIA* mRNA expression and the vehicle control group (GMEAN ± with geometric SD; ***P<0.001, **P<0.01 analyzed by one-way ANOVA).
- Similar reductions in human MMP7 protein and enzyme activity were observed in media supernatants. Data are normalized to vehicle control group (MEAN ± SD; *P<0.05, **P<0.01 analyzed by one-way ANOVA).

CONCLUSIONS

- **ARO-MMP7, currently in Phase 1/2a trials for the treatment of IPF, effectively silenced BAL MMP7 protein expression in non-human primates for over two weeks following a single inhaled dose.**
- **ARO-MMP7 potently silenced MMP7 expression and enzyme activity in human PCLS, highlighting the potential value of *ex vivo* systems for translational validation of RNAi drugs.**