Silencing MMP7 expression with a lung-targeted RNAi molecule limits fibrosis and preserves pulmonary function in bleomycin-injured rats

Tingting Yuan[1], Anthony Nicholas[1], Natalia Lakomski[1], Maria Afrazi[1], Holly Hamilton[1], Julia Hegge[1], James Hamilton[2], Tao Pei[1], Erik W. Bush[1]

1. Arrowhead Pharmaceuticals Inc., Madison, WI, USA; 2. Arrowhead Pharmaceuticals Inc., Pasadena, CA, USA

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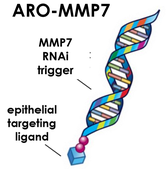
INTRODUCTION

- In idiopathic pulmonary fibrosis (IPF) aberrant basaloid cells overexpress matrix metalloproteinase 7 (MMP7), a secreted epithelial protease that promotes inflammation and fibrosis via cleavage of extracellular matrix proteins, receptors and cytokines
- MMP7 protein is a predictive biomarker of disease severity in IPF and other progressive fibrotic diseases like NASH and biliary atresia
- Genetic loss of *MMP7* function protects mice from pulmonary fibrosis. In man, gain-of-function *MMP7* alleles are linked to IPF
- Development of highly selective traditional small molecule active site inhibitor drugs has been challenging as MMP7 shares catalytic domain homology with dozens of other matrix metalloproteinase family members
- Therapeutic small interfering RNAs (siRNAs) offer an important novel approach to selectively silence challenging drug targets like *MMP7*

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

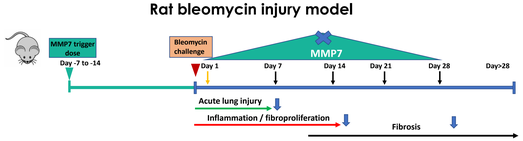


METHODS

Rat studies

- Animals received an RNAi trigger specifically targeting rat *MMP7* mRNA, which was administered by inhalation or multiple intratracheal (IT) instillations 1-2 weeks before bleomycin administration.
- Bronchoalveolar lavage (BAL) and lung tissues were collected at time points ranging from 1-4 weeks after bleomycin injury.
- *In vivo* respiratory functional mechanics were evaluated via FlexiVent.

Rat bleomycin injury model



Nonhuman primate study

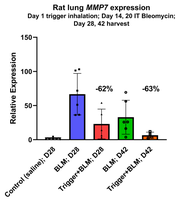
- After receiving baseline BAL collections 1 week prior to dosing, male cynomolgus monkeys received a single inhaled dose of either aerosolized vehicle (sterile isotonic saline) or ARO-MMP7 via a face mask exposure system. Pulmonary deposited doses were 0.24, 0.66, 1.10, and 1.71 mg/kg (n=3 animals per dose level).
- Two weeks after ARO-MMP7 exposure, post dose BAL and lung tissue (12 regional lung tissue samples per animal) samples were collected.
- Lung tissue samples were processed for *MMP7* mRNA and protein expression analysis by qRT-PCR and Western blot. BAL samples were processed for exosomal *MMP7* mRNA and protein expression analysis by qRT-PCR and Western blot.

Human precision cut lung slice (PCLS) study

- Fresh lung slices from a healthy donor were cultured and exposed to ARO-MMP7 (0, 0.1, 0.3 or 1 μM) for seven days then processed for analysis of *MMP7* mRNA expression by qRT-PCR.

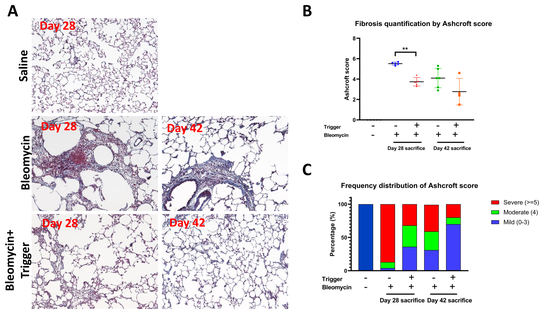
RESULTS

An inhaled RNAi trigger conjugate durably silences pulmonary *MMP7* mRNA overexpression in the rat bleomycin injury model



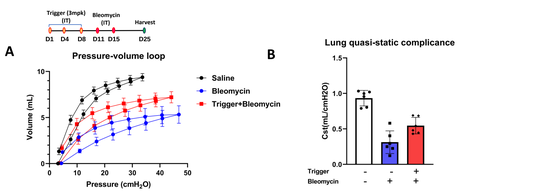
- Sprague Dawley rats received a single inhaled aerosol exposure of 1.4 mg/kg pulmonary delivered dose (PDD) saline vehicle or *MMP7*-targeting RNAi trigger conjugate on Study Day 1. Bleomycin (2U/kg) or saline was dosed intratracheally on Study Day 14 and 20.
- Lung tissues were collected on Study Day 28 and 42 for *MMP7* mRNA qRT-PCR (left) and histopathological analysis (below).
- *MMP7* expression was robustly induced by bleomycin injury
- RNAi trigger treatment significantly silenced bleomycin-induced *MMP7* overexpression measured 2 weeks (62% reduction) and 4 weeks (63% reduction) after injury.

MMP7 silencing limits pulmonary fibrosis in the rat bleomycin injury model



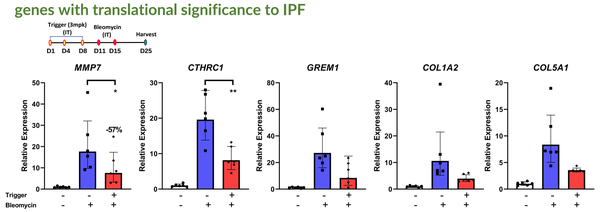
- **Panel A, above**: Left lung lobes were fixed in 4% PFA for Masson's Trichrome staining. Bleomycin injury produced prominent pulmonary fibrosis on Day 28. Partial resolution of fibrosis was observed on Day 42. A single inhaled dose of *MMP7* RNAi trigger on Day 1 reduced fibrosis at both time points.
- **Panels B & C, above**: Pulmonary fibrosis was quantified using blinded Ashcroft scoring (grades 0-8). Trigger treatment on Day 1 resulted in significantly lower scores after bleomycin injury on Day 28 (mean with SD; **P<0.01 analyzed by one-way ANOVA). Frequency distribution of the scores shows the injury levels including severe (≥5), moderate (4), and mild (0-3) of all images of the subpleural regions in 20 fields/rat.

MMP7 silencing preserves lung function in the rat bleomycin injury model



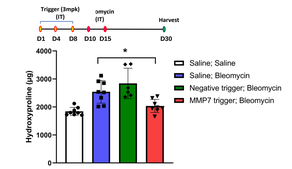
- Sprague-Dawley rats were treated intratracheally (IT) with either saline or a *MMP7*-targeting RNAi trigger (3 mg/kg) on Days 1, 4 and 8, followed by bleomycin (2 U/kg, IT) on Days 11 and 16. Lung function was evaluated on Day 25.
- Bleomycin injury significantly reduced lung compliance – which is a functional consequence of pathological fibrosis and tissue stiffening.
- *MMP7* silencing with RNAi trigger treatment limited bleomycin-induced reduction in lung compliance and preserved lung function.

MMP7 silencing in the rat bleomycin injury model limits lung expression of multiple genes with translational significance to IPF



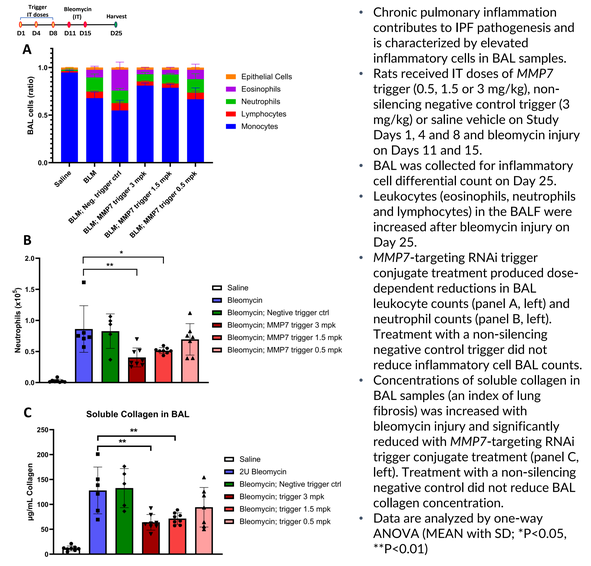
- A common set of upregulated marker genes (including *MMP7*, *CTHRC1*, *GREM1*, *COL1A2* and *COL5A1*) is shared between the rat bleomycin injury model and human IPF patients (Bauer 2015).
- Silencing *MMP7* expression with an RNAi trigger limited upregulation of multiple IPF marker genes in the rat bleomycin injury model.
- Data are normalized to *B2M* mRNA expression and the vehicle control group (GMEAN ± with geometric SD; *P<0.05, **P<0.01 analyzed by one-way ANOVA).

MMP7 silencing limits pulmonary collagen deposition in bleomycin-injured rats



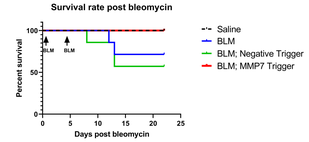
- Rats received IT doses of *MMP7* trigger conjugate, non-silencing negative control trigger conjugate or saline vehicle on Study Days 1, 4 and 8 and bleomycin injury on Days 10 and 15.
- On Day 30, middle and lower right lung lobes were collected for collagen content analysis by hydroxyproline assay.
- Increased lung collagen content associated with bleomycin injury was significantly attenuated by treatment with *MMP7*-targeting RNAi trigger conjugate. Treatment with a non-silencing negative control trigger did not reduce collagen content.

MMP7 silencing limits inflammation and BAL soluble collagen accumulation in the rat bleomycin injury model



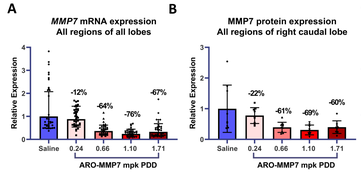
- Chronic pulmonary inflammation contributes to IPF pathogenesis and is characterized by elevated inflammatory cells in BAL samples.
- Rats received IT doses of *MMP7* trigger (0.5, 1.5 or 3 mg/kg), non-silencing negative control trigger (3 mg/kg) or saline vehicle on Study Days 1, 4 and 8 and bleomycin injury on Days 11 and 15.
- BAL was collected for inflammatory cell differential count on Day 25.
- Leukocytes (eosinophils, neutrophils and lymphocytes) in the BALF were increased after bleomycin injury on Day 25.
- *MMP7*-targeting RNAi trigger conjugate treatment produced dose-dependent reductions in BAL leukocyte counts (panel A, left) and neutrophil counts (panel B, left). Treatment with a non-silencing negative control trigger did not reduce inflammatory cell BAL counts.
- Concentrations of soluble collagen in BAL samples (an index of lung fibrosis) was increased with bleomycin injury and significantly reduced with *MMP7*-targeting RNAi trigger conjugate treatment (panel C, left). Treatment with a non-silencing negative control did not reduce BAL collagen concentration.
- Data are analyzed by one-way ANOVA (MEAN with SD; *P<0.05, **P<0.01)

MMP7 silencing limits mortality in the rat bleomycin injury model



- Rats received IT doses of *MMP7* trigger conjugate, non-silencing negative control trigger conjugate or saline vehicle on Study Days 1, 4 and 8 and bleomycin injury on Days 11 and 15.
- Bleomycin injury produced 28% mortality within two weeks which was completely prevented with *MMP7*-targeting RNAi trigger conjugate treatment.
- No survival benefit was observed with non-silencing negative control.

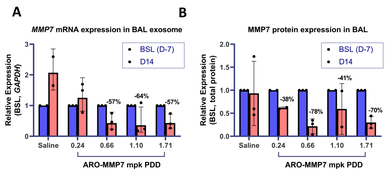
Dose-dependent silencing of pulmonary *MMP7* mRNA and protein in nonhuman primates two weeks after a single inhaled dose of ARO-MMP7



- Cynomolgus monkeys received a single inhaled aerosol exposure of ARO-MMP7 (0.24, 0.66, 1.1 and 1.71 mg/kg PDD) .
- Two weeks later, regional lung expression of MMP7 mRNA and protein was evaluated.
- Doses of ≥0.66 mg/kg ARO-MMP7 significantly reduced lung MMP7 mRNA and protein two weeks postdose.

A: qRT-PCR data normalized to GAPDH mRNA expression and the vehicle control group (GMEAN ± with geometric SD). N=36 regional lung samples per group (12 samples from each of 3 monkeys)
B: Western blot data normalized to total protein and the vehicle control group (MEAN ± SD)

ARO-MMP7 silences MMP7 protein and exosomal *MMP7* mRNA in nonhuman primate BAL samples two weeks postdose

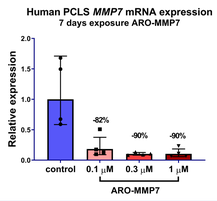


- Reduced BAL MMP7 protein (panel B, left) and exosomal *MMP7* mRNA (panel A, left) was observed at exposures of ≥0.66 mg/kg ARO-MMP7 and correlated with lung tissue protein and mRNA expression.
- BAL will be evaluated for evidence of ARO-MMP7 target engagement in future clinical trials.

A: qRT-PCR data normalized to baseline Day -7 and *GAPDH* mRNA expression in vehicle controls (GMEAN ± with geometric SD). n=2 for group 1; n=3 for group 2-5 (1 BAL sample per animal).
B: Western blot data normalized to baseline Day -7 and total protein expression in vehicle controls (MEAN ± SD). n=2 for group 2, 4; n=3 for group 1, 3, 5 (1 BAL sample per animal).

ARO-MMP7 silences *MMP7* mRNA expression in primary human lung tissue

- Human precision cut lung slices (PCLS) were exposed to ARO-MMP7 (0.1, 0.3 or 1 μM) for 1 week.
- Significant silencing of human *MMP7* mRNA was observed in all ARO-MMP7 treated groups.



CONCLUSIONS

- **In rats, inhaled epithelial-targeted RNAi triggers silence pulmonary *MMP7* overexpression, limit fibrosis and preserve lung function after bleomycin injury, effectively phenocopying findings in *MMP7* knockout mice and confirming the pathogenic role of MMP7.**
- **ARO-MMP7 silences pulmonary *MMP7* in nonhuman primates and in human lung slices and is a promising new therapeutic candidate for the treatment of IPF.**