Next generation miR-29 mimics as a therapy for pulmonary fibrosis

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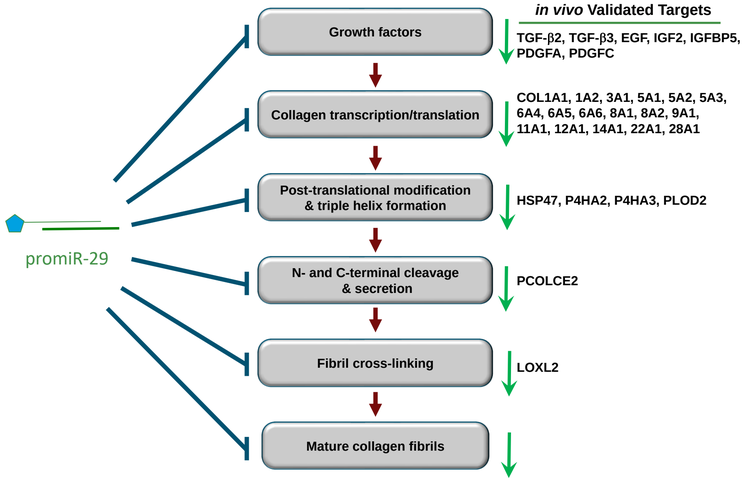
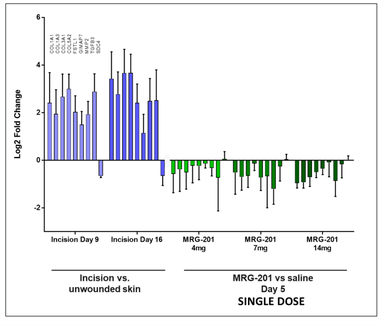
Abstract

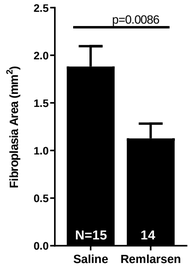
Rationale: miRNAs are small, non-coding RNAs that act as negative regulators of gene expression. The miR-29 family (miR-29a/b/c) targets multiple extracellular matrix proteins and profibrotic molecules, and is down-regulated during fibrotic disease, including idiopathic pulmonary fibrosis in humans. Current miR-29 mimics in clinical trials have demonstrated anti-fibrotic activity in humans but are not suitable for systemic disease due to instability.
Objective: To develop a stable, next-generation miR-29 mimic that can be administered parenterally at commercially viable doses with robust anti-fibrotic activity in the lung.
Methods: Numerous second generation miR-29 compounds with targeting capability and improved stability were assessed in vitro for retention of miRNA activity. Leads were advanced into mouse bleomycin studies, where bleomycin was given at day 0, and miR-29 mimics or controls were administered at 10 mg/kg I.V. at days 3, 7, and 10. At day 14, lung tissue was collected for RNA analyses by qRT-PCR. A therapeutic study was also performed in which bleomycin was given at day 0 and miR-29 mimics were administered at 10 mg/kg I.V. at days 10, 13, 17, and 20. Tissues were harvested at day 21 for molecular and histopathological analyses.
Results: Second generation miR-29 mimics retained miRNA activity in Normal Human Lung Fibroblasts in vitro as determined by assessing regulation of direct targets (COL1A1), downstream targets (ACTA2), and a broader array of fibrotic genes. Lead miR-29 compounds were subsequently assessed in the bleomycin model in vivo where the second generation miR-29 mimics with targeting capability and improved stability at 10 mg/kg I.V. showed down-regulation of myriad pro-fibrotic genes. Additional studies where a therapeutic dosing regimen was used showed that stabilized miR-29 mimics with a targeting conjugate at 10 mg/kg I.V. were able to robustly blunt pro-fibrotic response that resulted in less collagen deposition by quantitative histopathological analyses.
Conclusions: Second generation miR-29 mimics with targeting capability and improved stability retain miRNA activity and show efficacy in vivo in blunting the fibrotic response of bleomycin in mice. These next-generation miRNA mimics are efficacious at commercially viable dose levels not seen with earlier generation miRNA mimics.

miR-29 Family: Antifibrotic miRNAs

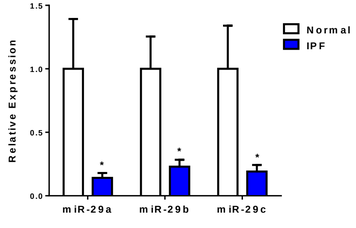


miR-29 Mimic Treatment Regulates Fibrotic Gene Expression and Significantly Blunts Fibroplasia in Skin of Human Subjects After Incisional Wounds



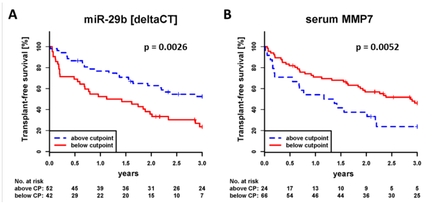


miR-29 Family is Markedly Reduced in Lungs of IPF Patients



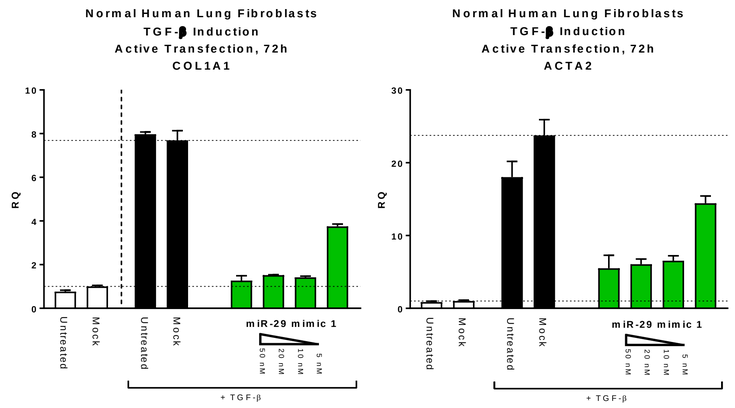
miR-29b and MMP7 in PBMCs Correlate with Survival in IPF

Variable	PBMC (Pittsburgh) cohort
n	94
Age [years]	68.0 ± 8.4
Gender, n [female/male]	26 / 68
Smoking status, *n* [ever/never]	59 / 35
FVC [% predicted]	64.9 ± 17.3
FEV1 [% predicted]	77.1 ± 19.8
DLCO [% predicted]	49.3 ± 18.3
BAL total cells [*10^6]	n. a.
Alveolar macrophages [%]	n. a.
Lymphocytes [%]	n. a.
Neutrophiles [%]	n. a.
Granulocytes [%]	n. a.



- De-identified PBMC samples from IPF patients in the “Pittsburgh cohort”
- All analyses done in the statistical software R
- “survMisc” was used to determine the optimal cut point for splitting cohort into low- and high-risk group, then plotted as Kaplan-Meier curves

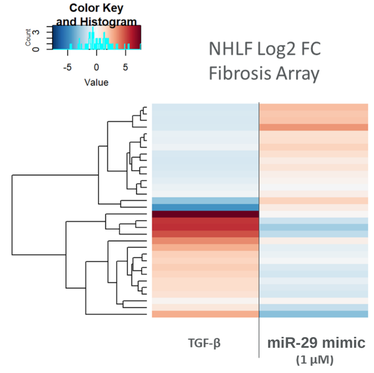
Next-Generation Stabilized, Conjugated miR-29 Mimics Retain Activity in NHLFs on Direct and Downstream Targets



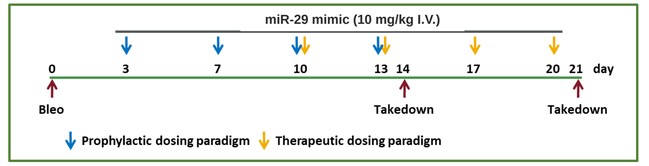
NHLF: Normal Human Lung Fibroblasts

Next-Generation Stabilized, Conjugated miR-29 Mimics Retain Activity in NHLFs Across a TGFβ-induced Fibrotic Signature

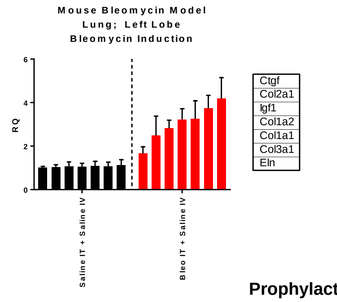
- Fibrosis Array assessed following TGFβ treatment in NHLFs, normalized to non-treated.
- miR-29 mimic treatment reverses this pro-fibrotic signature in NHLFs, normalized to TGFβ treatment

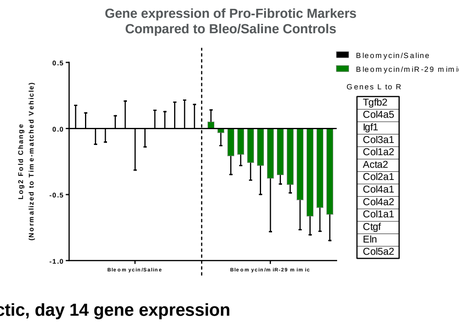


Design of Bleomycin Studies



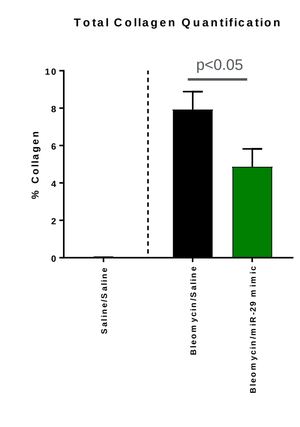
Stabilized, Conjugated miR-29 Mimic Down-Regulates Multiple Pro-Fibrotic Genes in Bleomycin-Treated Lungs

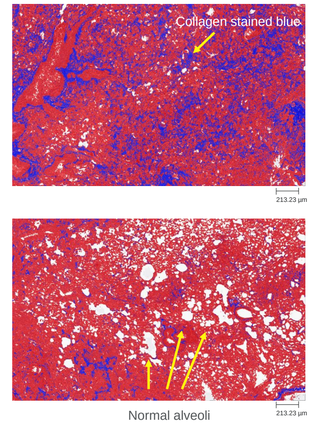




Prophylactic, day 14 gene expression

Stabilized, Conjugated miR-29 Mimic Significantly Blocks Pulmonary Fibrosis in Bleomycin-Treated Mice





Therapeutic, day 21 histopathology

Conclusions

- miR-29 mimics have demonstrated mechanistic proof-of-concept in humans
- miR-29 is down-regulated in IPF and inversely correlates with survival
- Next-generation stabilized, conjugated miR-29 mimics retain activity in NHLFs
- Stabilized, conjugated miR-29 mimics down-regulate pro-fibrotic genes from bleomycin-treated mouse lungs
- Stabilized, conjugated miR-29 mimics blunt pulmonary fibrosis by histopathology

Disclosures

RM, KR, SR, BW, AJ, and PR are employees and stock/option holders of miRagen Therapeutics, Inc.