Next Generation miR-29 Mimics as a Therapy for Pulmonary Fibrosis

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microRNA Therapeutics Regulate Systems Biology to Modify Disease

- microRNA-targeted therapy is focused on disease modification by restoring homeostasis to dysregulated processes
- microRNAs regulate complex biological systems
- microRNA-targeted therapies are intrinsically focused on disease-relevant pathways
- microRNA therapeutics particularly suited for complex, multigenic disorders
miR-29 is an Anti-Fibrotic miRNA

- Reduced Expression of miR-29 has been Implicated in the Development and Progression of a Wide Range of Fibrosis Indications

- miR-29 inhibits TGF-β activity, EMT, fibroblast-to-myofibroblast transition and ECM synthesis
- miR-29 inhibits every step of the collagen fibrillogenesis pathway

![Diagram showing various fibrosis indications and miR-29's role](image)

- Ocular fibrosis
- Pulmonary fibrosis
- Cardiac fibrosis
- Liver fibrosis
- Renal fibrosis
- Inflammatory bowel disease
- Osteoarthritis
- Dupuytren's contractures
- Cutaneous fibrosis

miRagen preclinical and/or clinical data + literature support

miRagen literature support
miR-29 Pathways and Systems Control

**Growth factors**
- TGF-β2, TGF-β3, EGF, IGF2, IGFBP5, PDGFA, PDGFC

**Collagen transcription/translation**
- COL1A1, 1A2, 3A1, 5A1, 5A2, 5A3, 6A4, 6A5, 6A6, 8A1, 8A2, 9A1, 11A1, 12A1, 14A1, 22A1, 28A1

**Post-translational modification & triple helix formation**
- HSP47, P4HA2, P4HA3, PLOD2

**N- and C-terminal cleavage & secretion**
- PCOLCE2

**Fibril cross-linking**
- LOXL2

**Mature collagen fibrils**

**TGF-β + Diseased ECM**

**Inflammation**

**miR-29**

*in vivo Validated Targets*
miR-29 Pathways and Systems Control

MRG-201 (promiR-29)

Growth factors
- TGF-β2, TGF-β3, EGF, IGF2, IGFBP5, PDGFA, PDGFC

Collagen transcription/translation
- COL1A1, 1A2, 3A1, 5A1, 5A2, 5A3, 6A4, 6A5, 6A6, 8A1, 8A2, 9A1, 11A1, 12A1, 14A1, 22A1, 28A1

Post-translational modification & triple helix formation
- HSP47, P4HA2, P4HA3, PLOD2

N- and C-terminal cleavage & secretion
- PCOLCE2

Fibril cross-linking
- LOXL2

Mature collagen fibrils

In vivo Validated Targets
A miR-29 Positive Feedback Loop in Fibrosis
**Therapeutic Hypothesis**

*MRG-201 restores homeostasis by modulating the positive feedback loops that maintain the fibrotic phenotype*
MRG-201 (miR-29 mimic)

Pathological Fibrosis & Tissue Repair

Skin  Lung  Liver  Eye
miR-29 as a Therapeutic in Cutaneous Fibrosis

Preclinical models

mPoC Human Volunteer Wound Repair

Drug  Placebo

Safety, PK, Target Engagement (PD)

Keloids

Hypertrophic Scars

Cutaneous Scleroderma

miRagen
Clinical Trial MRG201-30-001

Incision → Biopsy → Biopsy

Day 1 → Day 9 → Day 16

miR-29
qPCR
Nanostring

RNA

Normal wound healing consists of three overlapping phases:
- Inflammation (4 – 6 days)
- Proliferation (4 – 24 days)
- Remodeling (21 days – 2 years)
MRG-201 Mechanistic Proof-of-Concept in Human Incised Skin

- Evidence of PD activity (mPoC) after single administration of MRG-201
- Validation of preclinical PD biomarkers in human incised skin

![Log2 Fold Change Graph]

- Col1a1
- Col1a3
- Col3a1
- Col5a2
- Fstl1
- Gimap7
- Mmp2
- Tgfb3
- Sdc4

miRagen

Incision vs. unwounded skin

MRG-201 vs saline
Day 5
SINGLE DOSE
MRG-201 Treatment Significantly Blunts Fibroplasia in Human Incised Skin

![Graph showing the effect of MRG-201 treatment on fibroplasia depth, width, and area compared to saline. The graph indicates a significant blunting effect with a p-value of 0.0086.](image)
miR-29 as a Therapeutic in Cutaneous Fibrosis

Preclinical models

mPoC Human Volunteer Wound Repair

Safety, PK, Target Engagement (PD)

Keloids

Hypertrophic Scars

Cutaneous Scleroderma

miRagen
miR-29 Replacement in Pulmonary Fibrosis
miR-29 is Markedly Reduced in Lungs of IPF Patients
miR-29b and MMP7 in PBMCs Correlate with Survival in IPF

- 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
## MRG-201 Pharmacodynamic Biomarkers Translate to Multiple Fibrotic Indications

### Table of Expression Changes

<table>
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<th>MRG-201 antimiR-29</th>
<th>Day 9</th>
<th>Day 16</th>
<th>SSc skin vs. normal tissue</th>
<th>SSc lung vs. normal tissue</th>
<th>IPF lung vs. normal tissue</th>
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<td>Human skin incision vs. unwounded</td>
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- **TGFβ2**
- **Nedd4l**
- **Prickle1**
- **Faim2**
- **COL5A3**
- **Gimap7**
- **Cacna1g**
- **Colec11**
- **ELN**
- **Mfap2**
- **COL5A2**
- **COL1A1**
- **COL3A1**
- **COL11A1**
- **TGFβ3**
- **Fstl1**
- **COL1A2**
- **Cyt1**
- **MMP2**
- **Sdc4**
- **Sdc27**
- **Itgα3**
- **Numb**
- **Lbr**
miR-29 Mimic Represses Collagen Expression in Human IPF Fibroblasts and Epithelial cells *in vitro*
Optimizing miR-29 Replacement for Systemic Delivery to Lung

- The clinical miR-29 asset demonstrates anti-fibrotic activity in skin following local administration

- This asset is not amenable to systemic administration

- miRagen has developed additional miR-29 candidates that demonstrate systemic bioavailability delivery to lung
  - Medicinal chemistry optimization for enhanced stability
  - Targeting conjugates for delivery to tissues/cells of interest
Approach to Develop Next-Gen Mimics for Systemic Administration and Targeted Delivery

**In vitro Screening of Extensively Modified Mimics**
- Increase Nuclease Stability for Systemic Delivery
- Improve or Maintain potency
  - Measure of RISC loading

**Targeting conjugates**
- Tissue/indication specific receptor targeting ligands
- Lipophilic conjugates for improved uptake
- Evaluated *in vivo* and in specific cell type *in vitro*
Next-Generation Stabilized, Conjugated miR-29 Mimic Retains Activity in Normal Human Lung Fibroblasts (NHLFs) on Direct and Downstream Targets
Next-Generation Stabilized, Conjugated miR-29 Mimic Retains Activity in NHLFs Across a TGFβ-Induced Fibrotic Signature
Stabilized, Conjugated miR-29 Mimics Block Fibrosis in Human Precision-Cut Lung Slices

**Graphical Abstract**

- **Y-axis:** Collagen Content by Histology (au)
- **X-axis:** Conditions
  - CC 0h, CC 120h
  - FC 0h, FC 120h
  - C1 (1µM), C1 (5µM), C1 120h
  - C2 (1µM), C2 (5µM), C2 120h

- **Legend:**
  - **CC** = Control
  - **FC** = Fibrotic Cocktail
  - **C1** = Compound 1
  - **C2** = Compound 2

- **Statistical Significance:**
  - ***p < 0.001
  - ns = Not significant

- **Note:**
  - n = 3 (60 FOV each)
Outline of Bleomycin Studies

miR-29 mimic (10 mg/kg I.V.)

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↓ Prophylactic dosing paradigm ↓ Therapeutic dosing paradigm
Stabilized, Conjugated miR-29 Replacement Significantly Blocks Pulmonary Fibrosis in Bleomycin-Treated Mice

![Graph showing Total Collagen Quantification](image)

- **Saline/Saline**
- **Bleomycin/Saline**
- **Bleomycin/miR-29 mimic**

- **Collagen stained blue**
- **Normal alveoli**

**Bleomycin/Saline**

**Bleomycin/miR-29 mimic**

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$p<0.05$
Stabilized, Conjugated miR-29 Replacement Down-Regulates Multiple Pro-Fibrotic Genes in Bleomycin-Treated Lungs

Gene expression of Pro-Fibrotic Markers Compared to Bleo/Saline Controls

Log2 Fold Change (Normalized to Time-matched Vehicle)

-1.0

-0.5

0.0

0.5

1.0

Bleomycin/Saline

Bleomycin/miR-29 mimic

Tgfb2

Col4a5

Igf1

Col3a1

Col1a2

Acta2

Col2a1

Col4a1

Col4a2

Col1a1

Ctgf

Eln

Col5a2

Genes L to R
Stabilized, Conjugated miR-29 Replacement Reduces BALF IGF-1 and Serum TIMP1 in Bleomycin-Treated Mice
Subcutaneous Delivery of Stabilized, Conjugated miR-29 Replacement Shows Activity on Similar Gene-Set

Gene Expression of Pro-Fibrotic Markers Compared to Bleo/Saline

![Gene Expression Chart]

Thbs2, Ccl2, Col3a1, Plau, Col4a1, Col5a2, Plat, Igf1, Col1a1, Eln, Tgfb2, Col2a1, Cdh1, Tgfb3, Mfap2, Smad3, Ccr2, Egf, Ctgf, Acta2, Itgb6, Wnt11
Summary of Preclinical Data for miR-29 Replacement in IPF

- miR-29 is reduced in lungs of IPF patients and circulating miR-29 correlates with survival
- Next-generation stabilized and targeted miR-29 mimics retain activity and show anti-fibrotic activity in NHLFs and human precision cut lung slices
- Stabilized, conjugated miR-29 mimics block fibrosis in bleomycin-induced pulmonary fibrosis with increased potency compared to first generation miR-29 mimics
- Biomarkers identified in BALF and Serum for miR-29 mimic activity
- Stabilized, conjugated miR-29 mimics demonstrate activity by both intravenous and subcutaneous routes of administration
miR-29 Replacement in Hepatic Fibrosis
Hepatic Fibrosis Opportunities

- Myriad hepatic disorders and drugs result in hepatic fibrosis
  - Autoimmune hepatitis
  - Specific storage diseases and inborn errors of metabolism
  - Nonalcoholic steatohepatitis (NASH)
  - Primary biliary cholangitis (PBC)
  - Primary sclerosing cholangitis (PSC)
  - Disorders affecting hepatic blood flow
  - Mechanical obstruction
  - Drugs (Alcohol, Chlorpromazine, Methotrexate, Tolbutamide)

- Current therapies primarily rely on targeting hepatic inflammation
- Recent compounds in development have targeted single agents within the fibrotic pathway
- miR-29 is down-regulated in patients with hepatic fibrosis and miR-29 replacement has demonstrated anti-fibrotic effects in rodent models of fibrosis
miR-29 in Liver Fibrosis

- miR-29 is down-regulated in hepatic fibrosis in humans and rodent models
- Circulating miR-29 is inversely correlated with liver fibrosis in humans
- Hepatocyte-specific miR-29 knockout mice have exaggerated fibrosis when challenged

BDL: Bile Duct Ligation
Stabilized, Conjugated miR-29 Replacement Significantly Blunts Hepatic Fibrosis in CCl₄-Treated Mice
Stabilized, Conjugated miR-29 Replacement Down-Regulates Multiple Pro-Fibrotic Genes in CCl₄-Treated Livers

Gene expression of Pro-Fibrotic Markers Compared to CCl₄/Saline Controls

- Fstl1
- Col5a1
- Fbn1
- Col3a1
- Col1a2
- Col1a1
- Col4a1
- Plat
- Plau
- Col4a2

Log 2 Fold Change

CCl₄/Saline  CCl₄/miR-29 mimic
Internal Organ Fibrosis – Anticipated Path to IND

In vivo Models
- Bleomycin-Induced Pulmonary Fibrosis
- CCl₄-Induced Liver Fibrosis

Primary cell studies
- Phenotype and Molecular

Ex vivo Tissue Model
- Phenotype and Molecular

Non-GLP Toxicology/DRF/irritancy Rodent and NHP

IND-Enabling Toxicology, Clin Pharm

CMC: Bioanalytical method development and validation. Scale up synthesis – feasible for lead compound based on current discussions

Currently Initiating

IND/CTA
miR-29 PromiRs are Positioned for Myriad Fibrotic Indications

- Fibrosis is a major component of multiple organ failure, with a major effect on patient morbidity and mortality
- No approved anti-fibrotic compounds have a major impact on these debilitating diseases
- Decreased miR-29 levels correlate with advanced fibrosis and miR-29 is a key nodal point regulating pro-fibrotic gene expression
- miRagen has developed stable miR-29 drug candidate compounds that modulate these pro-fibrotic genes
  - Allows for a systems biology approach vs single agent in development
- miRagen has shown miR-29 mimics demonstrate anti-fibrotic effects
  - Efficacy shown in human skin and in rodent models of corneal, lung, and liver fibrosis
Acknowledgements

miRagen:
- Paul Rubin
- Aimee Jackson
- Corrie Gallant-Behm
- Kevin Rigby
- Linda Pestano
- Oliver Dansereau
- Shubh Roy
- Ben Werner

Yale:
- Maurizio Chioccioli
- Naftali Kaminski

Grants:
- NIH CADET II
  - 5UH3HL123886
- Contact: RLM@miragen.com