A MicroRNA-29b Mimic (MRG-201) Reduces Fibroplasia and Inhibits Collagen Expression in Skin Incisions in Normal Healthy Volunteers

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Abstract

OBJECTIVE/PURPOSE: Cutaneous scars, including hypertrophic scars and keloids, can arise from dermal injuries or surgery and represent a major unmet medical need, for which no approved drug therapies exist. MicroRNA-29 is well recognized as an anti-fibrotic miRNA that inhibits the expression of multiple collagens and other extracellular matrix genes in many tissues. miR-29 expression is repressed in cutaneous scars and keloids and expression of the miR-29 target genes COL1A1, COL1A2 and COL3A1 (collagens) are increased in those scars and in fibroblasts derived from those scars. Therefore, restoration of miR-29 expression in a skin wound or at the site of an excised scar/keloid is anticipated to lead to a therapeutic benefit by reducing scarring and/or preventing scar regrowth. The objective of this study was to test the safety and pharmacodynamics of such a miR-29-based therapeutic in normal healthy volunteers.

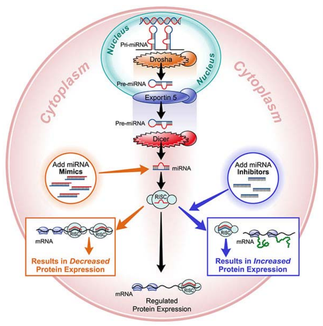
DESIGN: A novel oligonucleotide mimic of miR-29b (MRG-201) was evaluated in a Phase 1 placebo controlled double-blinded within-patient randomized single and multiple dose escalation clinical trial in 54 normal healthy volunteers with and without skin incisions. Safety, tolerability, pharmacokinetics and pharmacodynamics, including the effect of MRG-201 on skin histology and target engagement, were evaluated following intradermal administration of MRG-201.

RESULTS: MRG-201 was generally well tolerated at all dose levels, the maximum tolerated dose (MTD) was not determined. Local tissue exposure of MRG-201 was well above endogenous miR-29 levels while systemic exposure was low, supporting its favorable safety profile. Assessment of pharmacodynamic biomarkers demonstrated target engagement and repression of collagen mRNA expression following both single and multiple administrations of MRG-201 as compared to a placebo injected incision in the same subject. Fibroplasia was significantly reduced following multiple administrations of MRG-201 (mean placebo fibroplasia area 1.88mm^2, mean MRG-201 fibroplasia area 1.13mm^2, p=0.0086), and the magnitude of MRG-201 target repression corresponds to the impact on fibroplasia. Finally, MRG-201 did not adversely impact the healing process; there were no incidences of wound dehiscence.

CONCLUSIONS: These results demonstrate a favorable safety profile for MRG-201 and establish mechanistic proof-of-concept for MRG-201 in inhibiting collagen mRNA expression and preventing fibroplasia in skin incisions in normal healthy volunteers. These results support further investigation of MRG-201 as a novel therapeutic to inhibit cutaneous scar formation or prevent keloid or hypertrophic scar recurrence following excision.

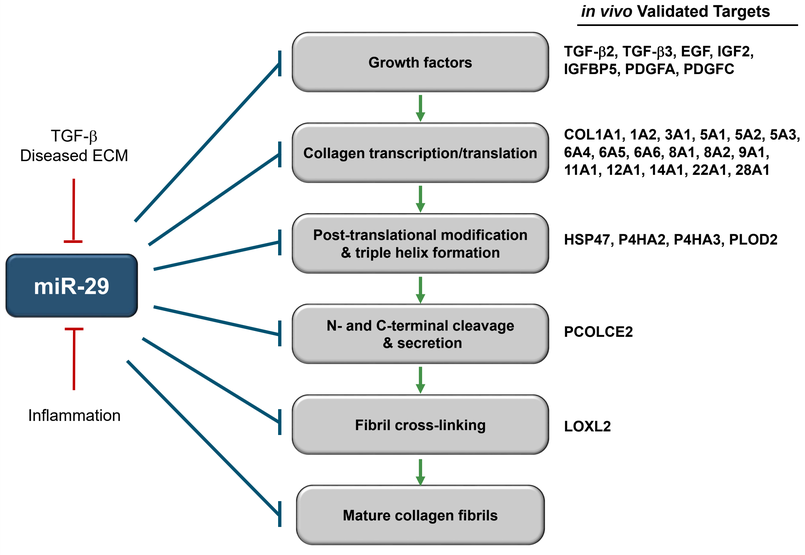
microRNAs

- microRNAs regulate complex biological systems and this role is amplified in disease states and biological stress.
- The pharmacology of microRNA-targeted therapies is intrinsically focused on disease relevant pathways.
- miRNAs serve as a "*molecular switch*" that constrains differentiation and maintains adaptive and maladaptive phenotypes.
- ***The unique objective of microRNA-targeted therapy is to achieve disease modification by restoring systems homeostasis.***



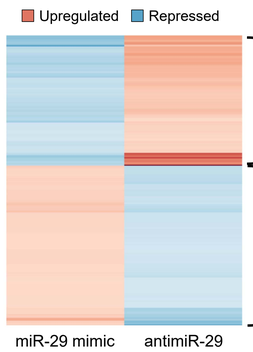
miR-29 Family: Antifibrotic miRNAs

hsa, mmu, rno-miR-29a: 5'-UAGCACCAUCUGAAAUCGGUUA-3'
hsa, mmu, rno-miR-29b: 5'-UAGCACCAUUUGAAAUCAGUGUU-3'
hsa, mmu, rno-miR-29c: 5'-UAGCACCAUUUGAAAUCGGUUA-3'
Seed sequence



Identification of miR-29 Target Genes *In Vivo*

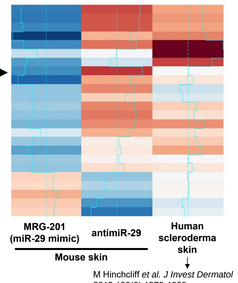
Reciprocally Regulated Genes



Functional Annotation (GO terms)

- ECM:
  - Collagen
  - Extracellular matrix
- Function:
  - Skin development
  - Epidermis development
  - Ectoderm development
  - Cellular homeostasis
- Adhesion/cell signaling
  - Signal peptide
  - Cell adhesion
  - Cation binding/transport
- Cell behavior
  - Cell differentiation
  - Apoptosis
- Structure:
  - Nuclear lumen
- RNA:
  - RNA processing
  - ncRNA processing
  - mRNA splicing
  - Helicase

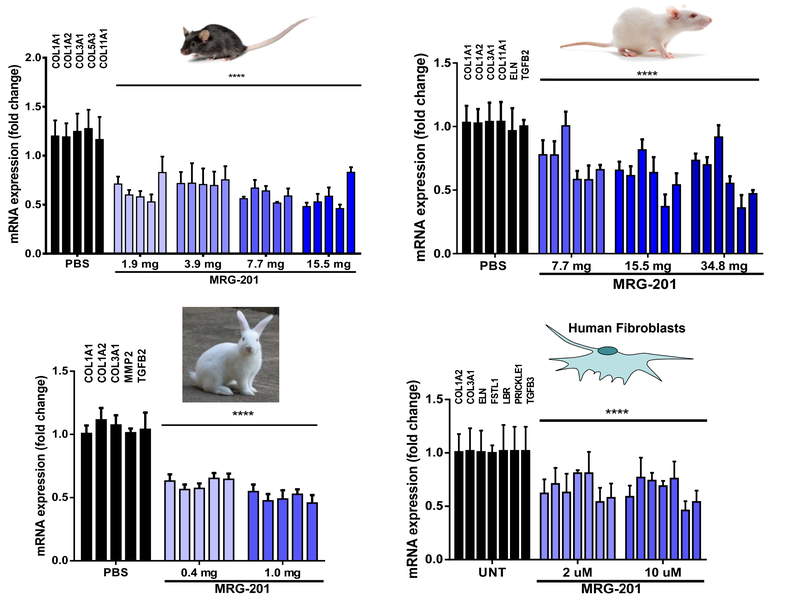
Selection of 24 Pharmacodynamic Biomarkers



M Hinchcliff *et al. J Invest Dermatol* 2013;133(8):1979-1989

MRG-201 PD biomarkers include:
Collagens
ECM molecules
Growth factors
Other factors associated with fibrosis

miR-29 Target Validation *in vitro* and *in vivo*

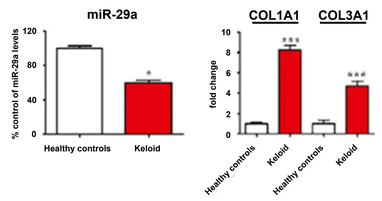


MRG-201 Target Genes are Conserved Across Multiple Species

Dysregulation of miR-29 Family Members and MRG-201 Target Genes in Keloids and Cutaneous Scars

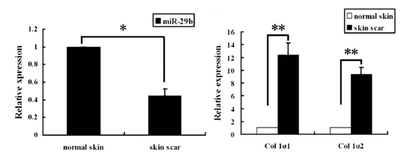
Keloids

G.-Y. Zhang *et al. Clin Exp Dermatol* 2016;41(4):341-345



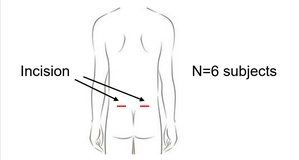
Cutaneous Scars

J. Cheng *et al. Am J Med Sci* 2013;346(2):98–103

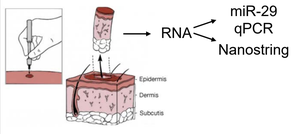


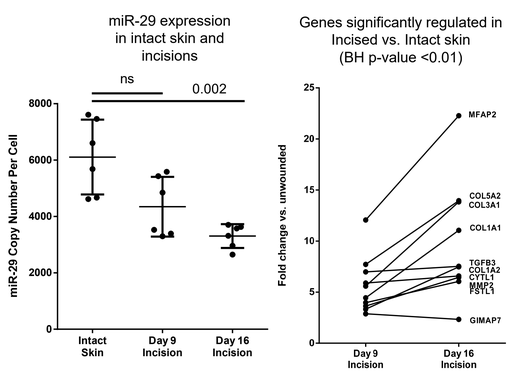
Clinical Trial MRG201-30-001

Part A: Kinetics of miR-29b Expression and Pharmacodynamic Gene Expression in Normal Healthy Volunteers with Sutured Skin Incisions



Biopsy: Intact skin
Incision Day 9
Incision Day 16
NO DRUG TREATMENT





miR-29b and MRG-201 Target Genes are Reciprocally Regulated With Skin Injury

Clinical Trial MRG201-30-001

Part B-D: Safety, Tolerability, Pharmacokinetics and Pharmacodynamics Assessment of MRG-201 Administered via Intradermal Injection in NHVs

Part B	Part C	Part D
Single Ascending Dose 0.5 – 14 mg Administered to Intact Skin	Single Ascending Dose 4 , 7, 14 mg Administered to Skin Incision	Multiple Ascending Dose 4, 7, 14 mg Administered M,W,F,M,W,F to Skin Incision
19 Subjects	9 Subjects	19 Subjects

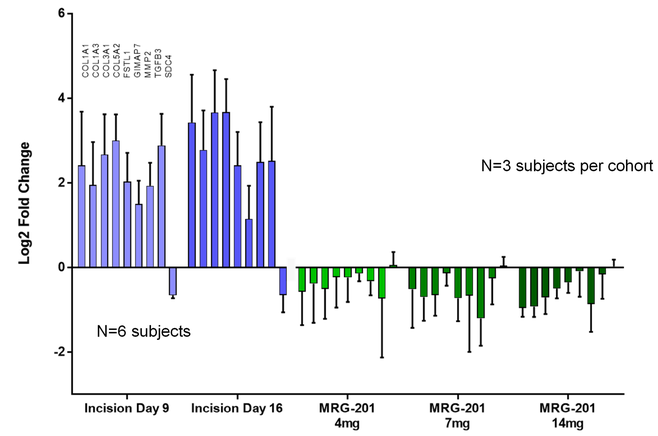
- Within-subject randomized double-blinded, placebo controlled trial: MRG-201 vs. saline
- 8 x 25 μL injections evenly spaced on both sides of a 1.5 cm line or incision
- MRG-201 is generally well tolerated at all doses. 11 injection site reactions of mild-moderate severity in 47 subjects (139 doses)
  - Injection site reactions not correlated with dose level or number of doses per subject
- "Possibly related" AEs: Headache (1), chills (1), fatigue (2), weakness (1), microscopic hematuria (1), sensation of warmth/tingling (2), pain at injection site (1) – all of mild severity (grade 1) and resolved
- **Maximum Tolerated Dose not determined.** Maximal deliverable dose = 14 mg / 200 μL

For more information about this clinical trial, Clinicaltrials.gov Identifier: NCT02603224

MRG-201 Pharmacokinetics and Tissue Biodistribution

- Low systemic exposure – most plasma samples tested BLOQ (10 ng / mL)
- T_{max} was variable, ranging from 15 minutes to 6 hours
- No evidence of plasma accumulation with repeated dosing
- Tissue levels not substantially higher after repeated dosing compared to single dosing

Validation of MRG-201 Pharmacodynamic Biomarkers

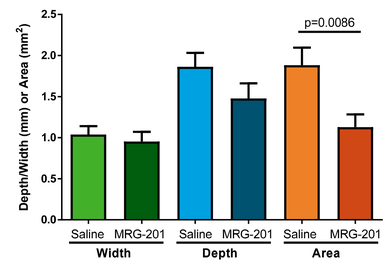


Incision vs. unwounded skin | **MRG-201 vs saline Day 5**

Part A - NO DRUG | **Part C – Single dose**

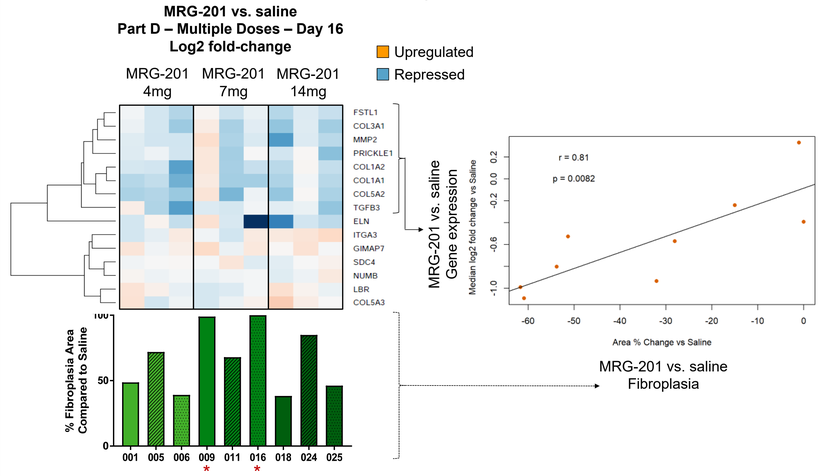
Evidence of Pharmacodynamic (PD) Activity and Mechanistic Proof of Concept (mPoC) After Single Administration of MRG-201

MRG-201 Treatment Significantly Blunts Fibroplasia in Human Incised Skin



N=16 subjects
(Additional 3 subjects did not have histology assessment performed)

H&E stain & assessment by a blinded pathologist



* These subjects had no or minimal detectable fibroplasia (<2mm^2) in either saline or MRG-201 treated incisions

MRG-201 Target Engagement Corresponds to Impact on Fibroplasia

Conclusions

- MRG-201 is well-tolerated in intact and incised skin
- No safety or injection site reaction concerns, no inhibition of wound healing
- No adverse histologic findings
- Maximum tolerated dose not defined; maximum deliverable dose = 14 mg / 200 μL
- Demonstrated mPoC for MRG-201 in human incised skin
- Fibrosis-associated pharmacodynamic biomarkers that are up-regulated following incision are down-regulated by MRG-201
- Magnitude of target engagement (repression of collagens and other factors) appears dose-proportional with single administration
- Within a subject, MRG-201 target engagement correlates with the impact on fibroplasia following multiple administrations