The miR-29b mimic remlarsen inhibits fibrosis of a corneal ulcer by preventing EMT and reducing profibrotic gene expression

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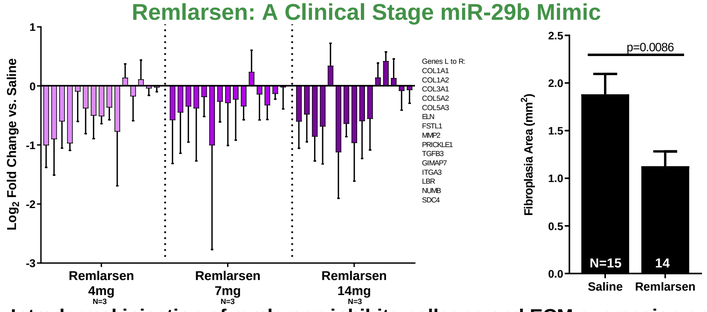
Abstract

Purpose: Anterior surface injury (e.g., trauma or burn) or corneal ulceration due to infection or neuropathy results in epithelial-to-mesenchymal transition (EMT), keratocyte activation, fibroblast-to-myofibroblast transition (FMT), and culminates in aberrant production of collagens and other extracellular matrix molecules. This process is termed corneal fibrosis and may lead to hazing and vision loss if located centrally or to irregular astigmatism and visual distortions if located peripherally. Few to no therapies are currently available to prevent or treat corneal fibrosis and this is therefore an area of high unmet medical need. miR-29b is a potent anti-fibrotic microRNA that inhibits EMT, FMT and collagen expression in multiple organs and tissues. This study investigated the use of remlarsen, a miR-29b mimic currently in clinical trials to inhibit pathologic cutaneous fibrosis, to prevent corneal fibrosis.
Methods: Remlarsen was administered topically to the rat cornea in the context of an alkali burn for up to 28 days (N=6-12 per group). Eyes were scored for corneal haze, then evaluated histologically for corneal thickness and expression of α-SMA, a marker of EMT and FMT. mRNA expression of collagens and other fibrosis-associated genes was assessed using quantitative RT-PCR. One or two-way ANOVA or non-parametric tests were used to assess statistical significance.
Results: Remlarsen treatment accelerated the healing of corneal alkali burns, resulting in a more rapid restoration of epithelial thickness and a reduction in stromal thickness as compared to saline treated burned eyes. Remlarsen treatment reduced the expression of multiple collagens and fibrosis-associated genes from 7-14 days post-burn and reduced α-SMA protein expression in the epithelium and stroma at 14 days. Furthermore, remlarsen treatment reduced corneal hazing and scarring beginning at 10 days post-burn. Dose and treatment schedule were optimized in preparation for IND-enabling toxicology studies.
Conclusions: Our results are consistent with remlarsen's mode of action as a potent antifibrotic in the context of tissue injury. In the rat alkali burn model, remlarsen accelerated repair and inhibited EMT, FMT, collagen mRNA expression and corneal hazing and scarring. These findings indicate that remlarsen may offer a novel therapeutic for prevention of corneal fibrosis following injury or ulceration.

miR-29 Family: Antifibrotic miRNAs

- miR-29a/b/c is widely believed to be antifibrotic; target genes include numerous growth factors, collagens, chaperones, processing enzymes, other ECM molecules, α-SMA
- Endogenous miR-29 expression is decreased following tissue injury and in fibrotic diseases in the lung, liver, kidney, heart, skin, colon, and the musculoskeletal system
- In the eye, reduced endogenous miR-29 expression is associated with Fuch's Endothelial Corneal Dystrophy, glaucoma, proliferative diabetic retinopathy, and age-related macular degeneration
- Corneal fibrosis can result from keratitis, trauma, chemical/thermal burns, or surgery and is an area of high unmet medical need
- miRagen Therapeutics hypothesizes that miR-29 mimics may prevent corneal fibrosis

Remlarsen: A Clinical Stage miR-29b Mimic



Intradermal injection of remlarsen inhibits collagen and ECM expression and inhibits fibroplasia at the site of iatrogenic skin wounds in normal healthy volunteers (Phase 1 clinical trial)

Methods

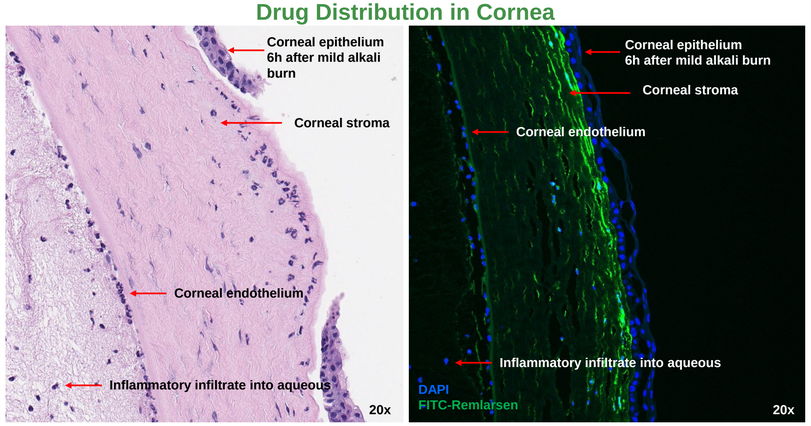
Corneal alkali burn

BID eye drops: Saline, Remlarsen

Analysis:

- Drug distribution
- PK
- Corneal morphology
- Corneal hazing
- α–SMA immunostaining
- Collagen and ECM mRNA expression

Drug Distribution in Cornea

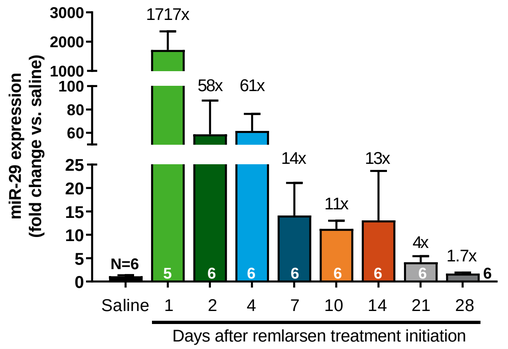


Remlarsen uptake into all layers of the cornea after alkali burn

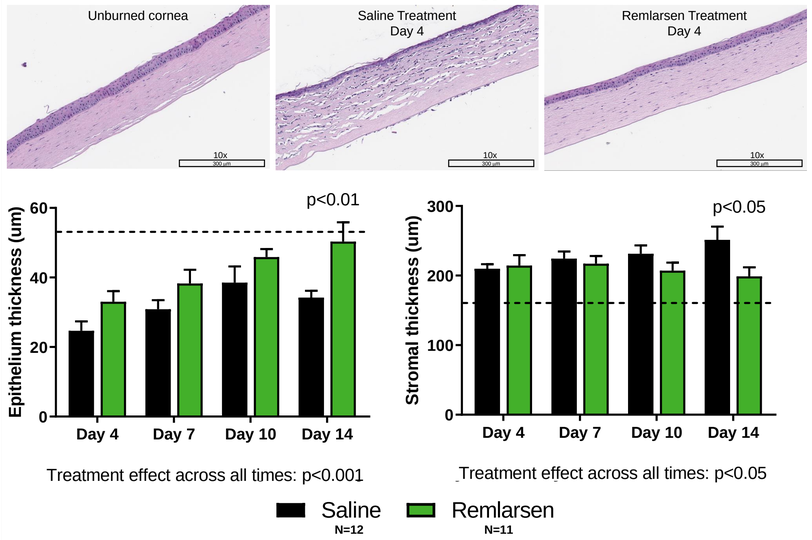
Remlarsen Pharmacokinetics

High levels of remlarsen uptake into cornea following corneal injury

Twice daily administration: Remlarsen uptake diminishes with re-establishment of corneal epithelium and tear film but remains above endogenous expression levels for ≥1 month

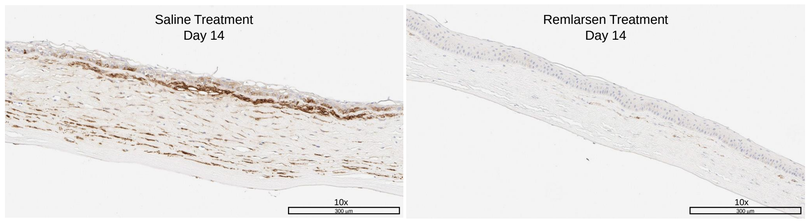


Corneal Histology



Remlarsen accelerates corneal wound healing

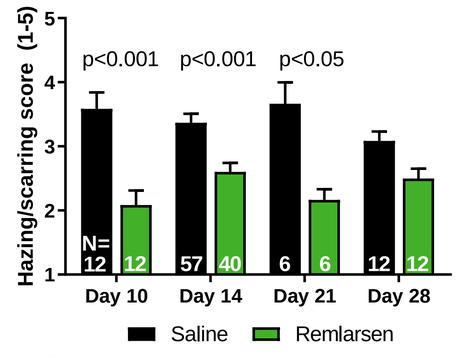
Immunohistochemistry for Alpha Smooth Muscle Actin



Remlarsen reduces EMT and FMT

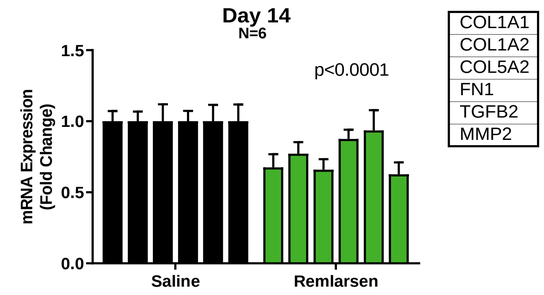
	Reduces inflammation	Accelerates healing	Reduces EMT	Reduces fibroblast activation	Reduces myofibroblasts	Inhibits collagen production
Remlarsen	●	●	●	●	●	●
Corticosteroids (SOC)	●	X		●		

Corneal Hazing/Scarring Assessment



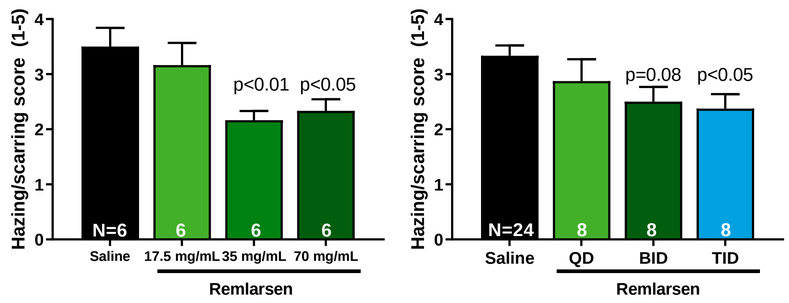
Remlarsen prevents corneal hazing/scarring

Remlarsen Pharmacodynamics



Remlarsen represses expression of TGF-β2 and multiple collagens
Also statistically significant at Day 7 and Day 10

Dose/Schedule Optimization



Optimal dose/schedule is 70 mg/mL remlarsen administered BID

Conclusions

- Remlarsen is taken up into injured cornea following topical drop administration
- Remlarsen pharmacokinetics is acceptable for treatment of corneal injuries/keratitis
- Remlarsen-treated corneas heal faster, with reduced scarring/hazing, reduced EMT
- miR-29 pharmacodynamic biomarkers are regulated *in vivo* in cornea by remlarsen
- Optimal remlarsen dose/schedule is 70 mg/mL BID
- Remlarsen may be an effective therapeutic to prevent fibrosis in the cornea in multiple diseases/conditions

Disclosures

- All authors are employees and stock/option holders of miRagen Therapeutics, Inc.