**Novel Anti-CTGF RNAi Therapy for Treatment of Proliferative Vitreoretinopathy (PVR) and Other Disorders**

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**Background**

Figure 1: Mechanism of Cellular Gene Silencing

RNA interference (RNAi) is a naturally occurring cellular process. Introduction of short double-stranded RNA (dsRNA) into cells can result in association with the RNA induced silencing complex (RISC) to target complementary mRNA sequences and degrade target genes.

**Abstract**

**Purpose:** Proliferative vitreoretinopathy (PVR) remains a serious medical problem despite advances in vitrectomy surgery. Connective tissue growth factor (CTGF) is overexpressed in human PVR and is believed to play a key role in development of cellular fibrosis. We have developed a new class of stable, self-delivering RNA compounds (sd-RxRNA) that incorporate features of both RNAi and antisense (and are spontaneously taken up by cells). Intraocular injection of the CTGF-targeting sd-RxRNA, RXI-109, results in robust, dose-dependent, long-lasting reduction of CTGF, without evidence of retinal detachment. Silencing of CTGF also impacts myofibroblast differentiation and collagen deposition, two key markers of fibrosis. RXI-109 is currently in Phase 1 clinical trials for derial scarring. Previously, we established mRNA silencing of CTGF in rodent retinas following intravitreal injection of a CTGF-targeting sd-RxRNA. Here we have evaluated the tissue distribution pattern of RXI-109 in vivo in retinal detachment models to spontaneous formation of subretinal gel scarring.

**Methods:** Previously, a CTGF-targeting sd-RxRNA administered by single intravitreal injection to Brown Norway rats resulted in a significant reduction of CTGF mRNA levels 48 h post injection relative to a control, non-targeting sd-RxRNA (52%, p<0.01). Here, intraocular administration of fluorescently-labeled RXI-109 (3 ug) in the presence and absence of retinal detachment was performed. Tissue distribution of RXI-109 and immunohistochemistry for various cell types was evaluated by confocal microscopy.

**Results:** Treatment with RXI-109 resulted in detectable uptake in all layers of the retina in both normal and detached retinas and, importantly, RXI-109 was detected through 14 days in the presence of a retinal detachment. Interestingly, an RXI-109 uptake was taken up by Müller cells, astrocytes and photoreceptors, all cell types involved in retinal scarring.

**Conclusion:** In the rat model of retinal detachment, RXI-109 was detected in all cell layers of the retina and was taken up by cell types known to be important to retinal scarring. This result, along with our previous report of specific and extended silencing of retinal genes by sd-RxRNAs, supports the potential use of an anti-CTGF treatment for PVR. Our next step is to evaluate the ability of RXI-109 targeting CTGF to reduce CTGF mRNA levels in vivo in the retinal detachment model and to assess the effect of RXI-109 on subretinal scar formation.

**Figure 2: sd-RxRNA Incorporates Advanced Features of RNAi and Antisense Technologies**

- Single stranded modified RNA compound
- No delivery formulation required
- Efficient cellular uptake and gene silencing
- Stability: stable, specific
- Reduced, long lasting in vivo efficacy in multiple species
- Biocompatibility/approved by FDA

**Figure 3: CTGF: A Central Factor in the Pathway to Fibrosis**

CTGF belongs to the CCN (CTGF/Cyr61/Coldl/Nov) protein family, which is comprised of six secreted proteins that reside in the extracellular matrix (ECM). By modulating signals from a variety of molecules, CTGF plays an important role in the ECM and influences a number of cellular processes critical for healing. These include reduction of cell adhesion, altered cell migration and proliferation. CTGF has also been shown to be essential for epithelial to mesenchymal transition (EMT), a process whereby normal epithelial cells are converted into myofibroblasts and subsequently produce scar tissue (which collagen is the major protein component). CTGF is over expressed in human PVR and is believed to play a key role in development of cellular fibrosis. Cellular responses to CTGF also have effects at the tissue level including remodeling, angiogenesis, changes in blood vessel architecture, and replacement of normal tissue with scar tissue. Selective interference of CTGF is expected to have anti-scarring and anti-fibrotic effects in dermal and other fibrotic indications.

**Figure 4: RXI-109 Silences CTGF Both in vitro and in vivo**

In vitro: Treatment of cells with RXI-109 by passive uptake results in potent gene silencing in vitro with ESO5 values in the nM range. A549 cells were treated with varying concentrations of sd-RxRNA for 48 hrs. CTGF mRNA levels were quantified using branched DNA assay (Affymetrix). Data were normalized to PPIB expression and plotted relatively non-targeting control (NTC) treated cells. Error bars represent standard deviation of biological triplicates. A 40 ug dose of CTGF-targeting or NTC sd-RxRNA was administered by intravitreal injection (5 ul) to rat eyes. Retinas were harvested 48 hours post injection and specific mRNA levels were quantified by qPCR. mRNA levels were normalized to a housekeeping gene (ß-actin) and graphed (+/- sd). For targeting vs. non-targeting control samples (NTC), p < 0.01; n=5 retinas for CTGF and NTC groups, n=5 retinas for PBS group.

**Figure 5: CTGF Targeting sd-RxRNA Silences CTGF in the Rodent Eye**

A) 40 µg dose of CTGF-targeting or NTC sd-RxRNA was administered by intravitreal injection (5 ul) to rat eyes. Retinas were harvested 48 hours post injection and specific mRNA levels were quantified by qPCR. mRNA levels were normalized to a housekeeping gene (ß-actin) and graphed (+/- sdev). For targeting vs. non-targeting control samples (NTC), p < 0.01; n=5 retinas for CTGF and NTC groups, n=5 retinas for PBS group.

**Figure 6: Retinal Uptake of Fluorescent RXI-109 in vivo in Normal and Detached Retinas in Rat**

A) 50 µg of fluorescently labeled RXI-109 (3 ug) was administered in 5 µl immediately after creating the retinal detachment. Confocal microscopy was performed 1 day and 14 days post administration. RXI-109 was detectable in all retinal layers 24 hours post administration both in the presence and absence of a detachment. At 14 days, RXI-109 was detectable primarily in the outer segments of the photoreceptors of detached retina (blue/vinculin stain Dapi)

**Conclusions**

- sd-RxRNA targeting CTGF silences CTGF in the rat retina
- In a rat retinal detachment model fl-RXI-109 is detectable in the retina through 14 days post injection
- fl-RXI-109 is taken up by cells known to be important in ocular scarring
- These results and our previous reports of specific and extended silencing of retinal genes support the potential use of sd-RxRNA for the treatment of PVR

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Figure 1: Silencing of CTGF in vitro and in vivo

- Treatments: Control, RXI-109 (red) were taken up by Müller cells (blue = anti-vimentin) in the retina 14 days post detachment and administration.

- rxRNA are asymmetric RNAi compounds containing a single duplex region (<15 base pairs) and a single-stranded phosphorothioated tail (≥ 6 nucleotides). In addition, sd-RxRNA compounds are chemically modified with stabilizing and hydrophobic modifications (e.g. sterics), which confer stability, reduced inflammatory response and efficient cellular uptake.