Development of sd-rxRNA® for Retinoblastoma Therapy

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OTC: RXII
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RNAi Overview

Targeting and Eliminating Disease Genes with sd-rxRNA

1. sd-rxRNA, designed to target a disease gene, is administered to a tissue

2. sd-rxRNA's drug-like properties enable efficient membrane penetration and accumulation in the cells

3. sd-rxRNA's structural and chemical modifications enable efficient loading into the RISC complex, where the two strands are split apart and a guide strand is retained within the RISC

4. Guide strand loaded RISC binds the target mRNA and cleaves it, blocking protein production and achieving a therapeutic effect
sd-rxRNA Combines Features of RNAi and Antisense Technologies

- **Medicinal Chemistry**
  - Improved cell uptake and PK/PD

- **Conventional RNAi**
  - Potent, long-lasting activity

- **Conventional Antisense**
  - Clinically relevant, validated PK/PD

- **sd-rxRNA**
  - Single compound designed to not require delivery vehicle
  - Robust uptake & silencing in multiple preclinical models
  - Structural diversity = novel intellectual property
  - Combining many positives of RNAi & antisense, while avoiding many negatives
  - Provides for broad pipeline of RNAi drugs for unmet medical needs

sd-rxRNA therapeutic compounds with drug-like properties
sd-rxRNA: Robust Cellular Uptake

*in vitro and in vivo*

Delivery and silencing demonstrated in many different cell types
Human, Primate, Rat, Mouse, Adherent, Non-adherent, Primary, Transformed

Efficient delivery of sd-rxRNA to multiple tissues *in vivo* upon local and systemic administration
sd-rxRNA: Improved Retinal Delivery vs. Stabilized RNAi Compounds

Intravitreal injection of fluorescently-labeled sd-rxRNA results in retinal delivery in mouse and rabbit.
sd-rxRNA: Extended Silencing in vivo in the Rodent Eye

- 3 µg PPIB or NTC administered by intravitreal injection (in 1 µl) to mouse eyes
- mRNA levels were quantified by Quantitative PCR (QPCR) and normalized to b-actin
- Data assembled from 5 different studies to enable sufficient 'n' for each data point (n=5-8); graphed +/- SD relative to PBS in each study; ** p ≤ 0.01
- PBS = Phosphate Buffered Saline (vehicle)
- NTC = Non-Targeting Control sd-rxRNA
- PPIB = Anti-cyclophilin B sd-rxRNA

Duration of Silencing Induced by PPIB Targeting sd-rxRNA

<table>
<thead>
<tr>
<th>Time after injection, days</th>
<th>PBS</th>
<th>NTC</th>
<th>PPIB</th>
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<tr>
<td>28</td>
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</table>
sd-rxRNA: Preliminary Safety Evaluation

Color Fundus Photography revealed no RPE degeneration or other overt signs of retinal damage post administration of sd-rxRNA or PBS.

- 5 µg (1 µl) of sd-rxRNA targeting MAP4K4 was administered by intravitreal injection to mouse eyes.
Fluorescein angiography revealed no leakage of retinal/choroidal blood vessels through 3 weeks post administration of sd-rxRNA or PBS.

- 5 µg (1 µl) of sd-rxRNA targeting MAP4K4 was administered by intravitreal injection to mouse eyes
- Images collected over the course of three weeks
Optical Coherence Tomography analysis revealed no changes in retinal morphology through 4 weeks post administration of sd-rxRNA or PBS.

- 5 µg (1 µl) of sd-rxRNA targeting MAP4K4 was administered by intravitreal injection to mouse eyes
- Images collected over the course of 4 weeks
ERG recordings reveal similar retinal function following dosing with sd-rxRNA or PBS.

- 5 µg (1 µl) of sd-rxRNA targeting MAP4K4 was administered by intravitreal injection to mouse eyes
- Recordings collected at baseline and three weeks post administration
sd-rxRNA: Dose Dependent Silencing *in vitro* in Retinoblastoma Cells

PPIB mRNA levels were reduced in a dose dependent manner relative to non-targeting control (NTC) sd-rxRNA forty eight hours post administration.

- 50,000 cells per well were treated with PPIB targeting sd-rxRNAs at 0.01, 0.025, 0.05, 0.1, 0.3, and 1 uM.
- At 48 hours, PPIB mRNA levels were quantified by a branched DNA assay.
Uptake of sd-rxRNA *in vivo* in Mouse Retina and Tumor Cells 24 hr Post Injection

**sd-rxRNA** Cone arrestin  sd-rxRNA  Cone arrestin

Twenty-four hours post injection

a) sd-rxRNA (red) co-localized with tumor cells (green) in the subretinal space

b) sd-rxRNA co-localized with tumor cells in the vitreous

c) sd-rxRNA is visible in the retina

- Mouse eyes were seeded subretinally with Y79 retinoblastoma cells
- 10 µg of DY547-labeled sd-rxRNA (red) was administered by intravitreal injection (1µl) 3 weeks after seeding
Summary

- sd-rxRNA: self-delivering RNAi compounds
  - Robust cellular uptake in the absence of any delivery vehicle
  - Dose-dependent target-specific silencing in vitro and in vivo
  - Full retinal penetration/delivery in vivo in the mouse and rabbit eye
  - Extended duration of effect (at least 14 days) following a single intravitreal injection in mouse
  - Preliminary safety evaluations show no morphological or functional changes to the eye following administration

- sd-rxRNAs exhibit dose-dependent target-specific silencing in Y79, RB176, and RB177 retinoblastoma cells in vitro

- sd-rxRNA is visible in tumor cells in the subretinal space and in the vitreous 24 hours post injection

Next steps
  - Design and characterize sd-rxRNAs against novel retinoblastoma targets
  - Evaluate specific sd-rxRNAs in human retinoblastoma cells in an orthotopic mouse xenograft model
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Poster: 6262 - D0070. Novel Anti-CTGF RNAi Therapy for Treatment of Proliferative Vitreoretinopathy (PVR) and other Ocular Disorders. Thursday 10:30-12:15