An Update on the Development of sd-rxRNA® for Retinoblastoma Therapy

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ARVO
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NASDAQ: RXII
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Program Number: 6020

Disclosure Block:

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. Conventional RNAi is potent and long-lasting, while conventional antisense is clinically relevant and validated PK/PD. Medicinal chemistry has improved cell uptake and PK/PD.

- 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

Keratinocytes human primary
ARPE-19 retinal pigment epithelium
SH-SY5Y neuroblastoma
Macrophages primary mouse
Hepatocytes primary mouse

Skin
Eye
Spinal cord
Alveolar macrophages
Liver
sd-rxRNA: Improved Retinal Delivery and Extended Silencing in vivo

Mouse

Immediately post dose

24 h

24 h

Rabbit

24 h

Twenty-four hours post injection:

- Fluorescently-labeled sd-rxRNA detected in all retinal layers in mouse and rabbit
- sd-rxRNA treatment results in statistically significant reduction of target-specific mRNA levels for weeks.
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 48 hours post administration.

- Model sd-rxRNAs were designed to target PPIB, a ubiquitously expressed gene.
- 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

**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
MDM2 Targeting sd-rxRNA

- Murine double minute gene 2 (MDM2)
- Negative regulator of p53 tumor suppressor pathway
- Increased levels inactivate p53 and prevent its tumor suppressor functions
- Highly expressed in retinoblastomas*
- Required for retinoblastoma cell proliferation and survival*

*Xu et al., 2009. Cell 137: 1018.
MDM2 Targeting sd-rxRNA Selection

Designed 25 compounds based on algorithm with multiple selection criteria and tested \textit{in vitro} in RB177 cells in three-point dose-response studies

Identified 7 compounds with dose-dependent reduction of MDM2 mRNA levels

Selected 4 compounds and performed six-point dose response studies in both RB177 and RB176 cells

Identified two compounds to further evaluate \textit{in vitro} and \textit{in vivo}
MDM2 Targeting sd-rxRNA Selection in RB177 cells

NTC = non targeting control sd-rxRNA
Identified 7 compounds with dose-dependent reduction of MDM2 mRNA to further evaluate.

Selected 4 compounds and performed six-point dose response studies in both RB177 and RB176 cells.

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NTC = non targeting control sd-rxRNA
Selected 4 compounds and performed six-point dose response studies in both RB177 and RB176 cells.
MDM2 sd-rxRNAs Significantly Reduce MDM2 mRNA *in vitro* Through Day 6

- 50,000 cells per well were treated with MDM2 targeting sd-rxRNAs
- MDM2 mRNA levels were quantified by a branched DNA assay
MDM2 sd-rxRNAs Significantly Reduced MDM2 Protein Levels \textit{in vitro}

2-day Post Incubation

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4-day Post Incubation

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Summary

- sd-rxRNA: self-delivering RNAi compounds
  - Robust cellular uptake in the absence of any delivery vehicle with dose-dependent target-specific silencing *in vitro* and *in vivo*
  - Extended duration of effect (at least 14 days) following a single intravitreal injection in mouse

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

- sd-rxRNAs targeting MDM2 designed and screened *in vitro*

- Two compounds selected from screen that exhibit dose dependent reduction of MDM2 mRNA levels *in vitro*

- MDM2 protein levels were also reduced following treatment *in vitro*

- Next steps:
  - Evaluate impact on cell proliferation *in vitro*
  - Evaluate MDM2 sd-rxRNAs in human retinoblastoma cells in an orthotopic mouse xenograft model
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