QR-1123 prevents retinal degeneration in humanized adRP models through allele-specific knockdown of P23H rhodopsin mRNA

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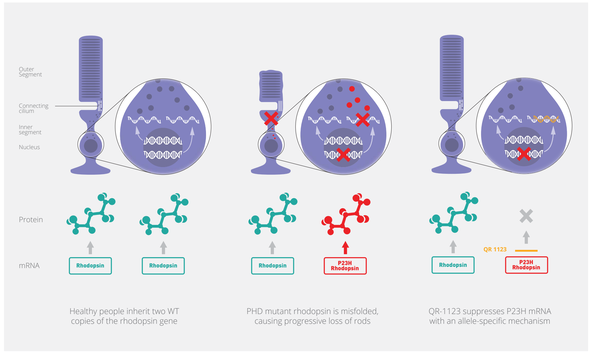
Introduction

- Autosomal dominant retinitis pigmentosa (adRP) is a group of rare inherited eye disorders causing photoreceptor degeneration that leads to progressive vision loss. adRP is characterized by progressive deterioration of rod photoreceptor function, as assessed by diminished dark adapted electroretinogram (daERG) and perimetry responses, followed by the loss of cone photoreceptors.
- The c.68C>A mutation of the rhodopsin (*RHO*) gene, resulting in a proline-to-histidine (P23H) substitution in the RHO protein, is the most common mutation associated with adRP. Due to a founder effect of a common ancestor, adRP resulting from the P23H *RHO* mutation is present mainly in a population of Americans of Western European origin, and is almost exclusive to the United States. Although accurate prevalence figures do not exist, the number of patients in the US is estimated to be 2,500-3,000.
- QR-1123 is an allele-specific, RNase H1 activating antisense oligonucleotide (AON) that selectively knocks down the *RHO* mRNA with the P23H mutation. This selective inhibition of the mutant allele, preserves expression of the wild-type (WT) variant, and results in an increased function of WT RHO protein in photoreceptors.



The P23H mutation in one of the copies of the *RHO* gene leads to adRP. The mutated RHO protein causes progressive degeneration of photoreceptor cells through dominant-negative effects by decreasing the function of WT protein. QR-1123 binds to and degrades the mutated P23H *RHO* mRNA in a sequence-specific manner, but has no effect on the WT transcript. By reducing the amount of mutant *RHO* in the eye, QR-1123 is predicted to augment WT *RHO* mRNA expression levels, and lead to restoration of photoreceptor function and reversal of disease phonotype.

Objectives

- **To assess the ability of QR-1123 to elicit allele-specific knockdown of P23H rhodopsin mRNA**
- **To study the potency of QR-1123 in rescuing molecular and phenotypic defects in various adRP models**

Materials & Methods

HEK293 cells stably expressing human WT or P23H rhodopsin mini-genes were generated as described in Lima et al., 2014. QR-1123 was introduced to the cell lines by electroporation. Human P23H/WT transgenic mice (hP23H/WT Tg) received one microliter of PBS or AON through intravitreal (IVT) injections. Eyes were sectioned through the optic nerve head and H&E stained for Outer Nuclear Layer (ONL) analysis. Quantification of *RHO* WT and P23H mutant mRNA was performed using qRT-PCR assays. For the Non-Human primate QR-1123 selectivity study, animals received vehicle (PBS) or QR-1123 at a dose level of 0, 150, 500, 600, or 750 µg. For the proof-of-concept study in non-human primates, animals received vehicle (PBS) or QR-1123 surrogate (targeting human/cynomolgus wild-type rhodopsin) by IVT injection at a dose level of 0, 50, 100, 200, 400, or 600 µg. Quantification of *RHO* WT and P23H mutant mRNA for both studies was performed using qRT-PCR assays. For the Non-Human primate pharmacokinetic study, QR-1123 tissue concentrations are measured using hybridization ELISA.

Results

Allele-specific knockdown of P23H mutant mRNA by QR-1123

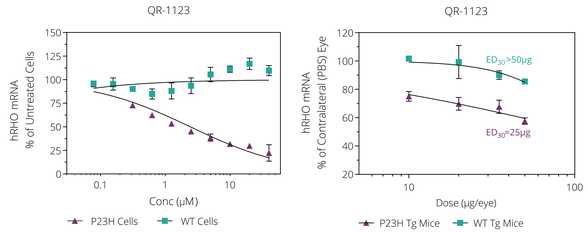


Figure 1 Over 200 AONs were tested and optimized for selective activity to the P23H RHO mRNA over WT RHO mRNA. QR-1123 was selected based on its potency and capacity to deliver allele-specific knockdown of P23H RHO mRNA both in vitro (HEK293 cells expressing WT or P23H human rhodopsin mini-genes) and in vivo (Human P23H/WT transgenic mice). In epithelial cells, QR-1123 selectively reduced the levels of RHO mRNA in P23H cells (IC50 = 2.4 µM), but had no detectable effect in WT cells (IC50 > 40 µM). In the transgenic mice model, QR-1123 elicited an allele-specific effect over P23H RHO (ED30=25 µg) but had no detectable effect in WT RHO (ED30 > 50 µg).

QR-1123 Allele-specific knockdown further confirmed in cynomolgus monkey

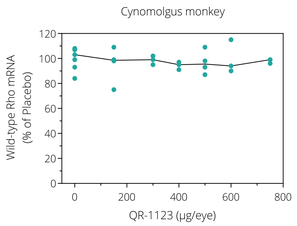


Figure 2 Levels of monkey WT *RHO* mRNA were measured by qRT-PCR 14 days after IVT injections. The *RHO* levels of individual animals were normalized to *CRX* mRNA levels. Values are presented as % of mean of the vehicle group.

QR-1123 treatment did not result in significant fluctuations in WT RHO mRNA levels or any of the other transcripts studied in the cynomolgus monkey, underlining the pharmacological selectivity of this molecule. Besides confirming specificity of QR-1123 for the P23H mRNA, these results indicate lack of undesirable secondary effects due to interaction at the WT level.

Long half-life of QR-1123 in cynomolgus monkey retina provides for infrequent IVT dosing

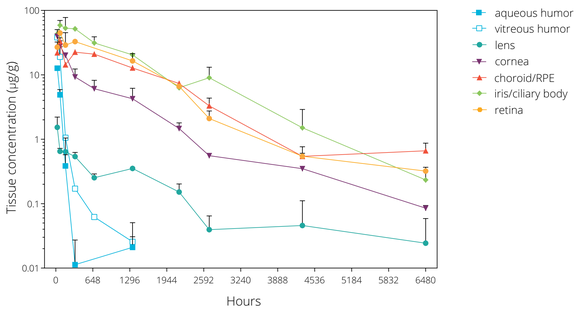


Figure 3 Mean Concentration Profiles (logarithmic) of QR-1123 in cynomolgus monkey ocular tissues following 150 µg/eye of intravitreous administration. Mean tissue half-life ranged between 385 hours (16 days) in vitreous humor to 1440 hours (60 days) in retina.

Proof-of-concept of *in vivo* target engagement in cynomolgus monkey using surrogate AON

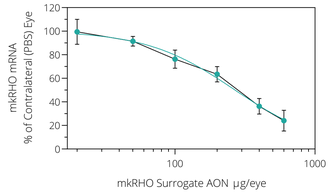


Figure 4 Effects of QR-1123 surrogate on cynomolgus monkey *RHO* mRNA after a single IVT injection. The QR-1123 surrogate (50-600 µg), or PBS were administered by IVT injection. *RHO* mRNA levels were measured 6 weeks later. The mRNA samples were normalized to *CRX* mRNA levels and presented as % of contralateral eye receiving PBS. Values are mean ± SD.

As the intended target of QR-1123, P23H RHO mRNA, is not present in the transcriptome of WT cynomolgus monkey, the surrogate molecule was used to evaluate the ability to reduce rhodopsin levels *in vivo* by RNase H1 cleavage of RHO mRNA, QR-1123's mechanism of action. The AON surrogate shows a clear dose response, with knockdown values of up to 76% when compared to PBS treated eyes. These results indicate that rhodopsin levels can be specifically reduced by AON-induced RNase H1 mediated cleavage of *RHO* mRNA.

Treatment with QR-1123 has a protective effect over anatomical and functional parameters in adRP mouse model

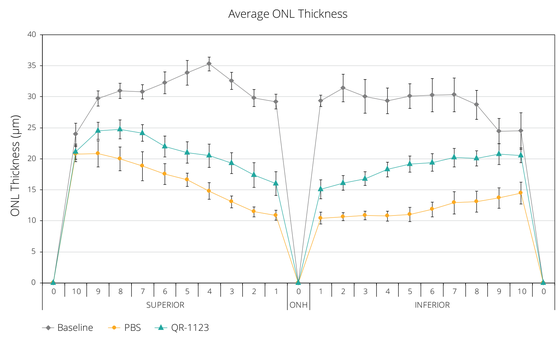


Figure 5 The ONL was quantitated from histological slides; 10 loci were selected in the superior and inferior regions of each mouse. Shown are the average values from 4 mice at Baseline and 7 mice treated with QR-1123 (± SEM). Two-tailed t test; **p < 0.01, ****p < 0.0001.

The hP23H Tg animals provided an important tool to estimate the effects of IVT AON injections in a biological context, and established a highly robust and specific response of QR-1123 to its intended target. The transgenic mouse model of adRP also allowed for a morphological analysis of the retina. Mice expressing the human P23H RHO mutation display progressive degeneration of photoreceptor cells, similarly to that observed in human patients but with a more aggressive time course. Single IVT injections of QR-1123 were sufficient to elicit a therapeutic response in the animals as compared to vehicle-treated controls. These results provide evidence for the mechanism of action of QR-1123 in selectively targeting the P23H rhodopsin mRNA, and alleviating adRP disease progression.

Conclusion

We have demonstrated the capacity of QR-1123, an investigational therapy for adRP, to selectively reduce human P23H rhodopsin expression and prevent retinal degeneration in adRP models.

- **In transfected cell lines containing mini-genes, QR-1123 selectively targets the human P23H mutant rhodopsin RNA through an allele-specific mechanism, without affecting the human WT rhodopsin mRNA levels**
- **Intravitreal dosing of QR-1123 into humanized knock-in mice expressing either WT or mutant rhodopsin selectively reduces the P23H human rhodopsin mRNA**
- **In humanized knock-in mice, QR-1123 reduced the rate of photoreceptor degeneration in both inferior and superior ONL**
- **In non-human primates, QR-1123 displayed allele-specificity and long half-life after IVT dosing**

Taken together, these results indicate that treatment with QR-1123 is a potentially effective therapy for adRP resulting from the P23H mutation in the rhodopsin gene.

Literature

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Acknowledgements

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