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Evaluation of AAV2tYF-GRK1-RPGR vectors in a canine model of *RPGR*-XLRP

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INTRODUCTION

X-linked retinitis pigmentosa (XLRP) caused by mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene is a primary rod and cone disease affecting ~20,000 patients in the US and EU.[1, 2] Gene replacement therapy using adeno-associated virus (AAV) vectors for gene delivery is effective in preventing photoreceptor degeneration and preserving retinal structure and function in disease animal models .[3, 4, 5]

Important considerations in the design and evaluation of an AAV vector include the capsid, promoter, cDNA, and the species of animals.[6] The RPGR cDNA contains a long purine-rich repetitive sequence in the ORF15 exon that is unstable during recombinant DNA manipulation. This complicates efforts to develop AAV-based vectors for gene therapy of XLRP caused by RPGR mutations. AAV5-hRPGRstbl vectors containing a stable human RPGR cDNA with multiple small deletions and nucleotide changes driven by either IRBP or GRK1 promoters have provided long-term structural and functional rescue in XLPR2 dogs.[3] A direct comparison of AAV5-GRK1-hRPGRstbl and AAV5-GRK1-hRPGRco, a vector containing an AGTC proprietary human RPGR cDNA (hRPGRco), indicated that both vector constructs were well tolerated and provided both structural and functional rescue for photoreceptors and bipolar cells in the treated retinal area in XLPRA2 mutant dogs subretinally injected with a single dose at an early stage of disease (~5 weeks of age).[7] Here we present safety and efficacy results of a non-GLP preclinical comparative dose range finding study in XLPRA2 dogs treated at mid-stage disease (~12 weeks of age with approx 40% lose of photoreceptors) with one of 3 dose levels of vectors expressing RPGRco or RPGRstb driven by a GRK1 promoter and packaged in AAV2tYF capsids.

METHODS

AAV2tYF-GRK1-hRPGRco and AAV2tYF-GRK1-hRPGRstb were produced using an HSV-based manufacturing system and purified by column chromatography. A positive control vector, AAV5-GRK1-hRPGRco, was produced using a plasmid transfection method and purified by iodiaxol gradient centrifugation. The final formulation of all vectors was in balanced salt solution (BSS) containing 0.014% Tween-20.

Two dogs per group received a 0.15 mL subretinal injection of AAV2tYF-GRK1-hRPGRco in the right eye and AAV2tYF-GRK1-hRPGRstb in the left eye at each of 3 dose levels (1.2×10^{11}, 6×10^{11} or 3×10^{12} vg/mL). One dog received the mid-dose of AAV5-GRK1-hRPGRco in both eyes (positive control, PC) and 1 dog received vehicle in both eyes (negative control, NC). Assessments included clinical ophthalmic examination performed at 3 days pre-dosage and Day 1, Day 3, Day 7, Day 14, Week 4 and Week 8 post-dosage, titration of antibodies directed against RPGR by enzyme-linked immunosorbent assay (ELISA) and antibodies directed against AAV2 by a neutralization assay. Photoreceptor rescue was determined by histology/immunohistochemistry (IHC) analysis of retinal tissues at termination for preservation of retinal structure and corrections of markers of disease, such as opsin mislocalization and Müller cell gliosis.

The study design is summarized in **Table 1**.

Table 1 Design of the XLPRA2 Dog Dose Range Finding Study

Group	# of Dogs	Vector		Conc. (vg/mL)	Dose (vg/eye)	Vol. (µL)
		Right Eye	Left Eye			
High-dose	2	AAV2tYF-GRK1-hRPGRco	AAV2tYF-GRK1-hRPGRstb	3.0×10^{12}	4.5×10^{11}	150
Mid-dose	2	AAV2tYF-GRK1-hRPGRco	AAV2tYF-GRK1-hRPGRstb	6.0×10^{11}	9.0×10^{10}	150
Low-dose	2	AAV2tYF-GRK1-hRPGRco	AAV2tYF-GRK1-hRPGRstb	1.2×10^{11}	1.8×10^{10}	150
PC	1	AAV5-GRK1-hRPGRco	AAV5-GRK1-hRPGRco	6.0×10^{11}	9.0×10^{10}	150
NC	1	Vehicle (BSS/0.014% Tween 20)	Vehicle (BSS/0.014% Tween 20)	N/A	N/A	150

RESULTS

No sustained signs of ocular discomfort or ophthalmic (including retinal) complications were noted in any of the eyes injected with the mid- or low-doses of AAV2tYF-GRK1-hRPGRco or AAV2tYF-GRK1-hRPGRstb, AAV5-GRK1-hRPGRco (positive control), or vehicle (negative control). Funduscopic examination at 8 weeks post-dosage showed signs of retinal detachment with signs of retinal inflammation in the eyes injected with the high-dose of either AAV2tYF-GRK1-hRPGRco and AAV2tYF-GRK1-hRPGRstbl .

The natural history of the RPGR/XLPRA2 disease shows that there is a slow decline in ONL thickness in RPGR/XLPRA2 dogs between 12 and 20 wks of age. As a result, examination of H&E-stained cryosections from comparable injected regions showed no obvious changes in ONL thickness between any treatment groups (Figure 1).

IHC analysis showed RPGR expression in photoreceptors with an apparent dose relationship. The levels of RPGR expression in eyes injected with AAV2tYF-hRPGRco were substantially higher than that in the contralateral eyes injected with AAV2tYF-hRPGRstb at the same dose levels (**Figure 1**) .

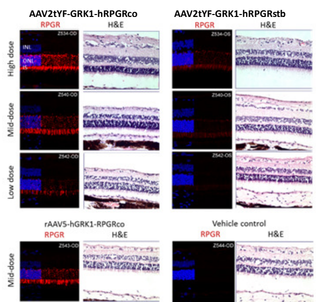


Figure 1 RPGR protein expression (in red) detected by IHC and retinal morphology by H&E staining in eyes injected with rAAV2tYF-hRPGRco (right eye) or rAAV2tYF-hRPGRstb (left eye) at concentrations of 3×10^{12} vg/mL (High-dose), 6×10^{11} vg/mL (Mid-dose) or 1.2×10^{11} vg/mL (Low-dose). The bottom images are from eyes injected with the positive control vector AAV5-GRK1-hRPGRco at a concentration of 6×10^{11} vg/mL (Mid-dose) or eyes injected with the negative control (BSS/0.014% Tween 20). The location of the inner nuclear layer (INL) and outer nuclear layer (ONL), shown in blue, and the inner segment of photoreceptors (IS), are also indicated.

In eyes treated with either AAV2tYF-GRK1-hRPGRco or AAV2tYF-GRK1-hRPGRstb at the high- and mid-doses, there was reduced disorganization of cone inner segment (CIS) membranes. IHC showed correction of rod opsin mislocalization (**Figure 2**) and middle/long wavelength (M/L) opsin mislocalization (**Figure 3**) in all AAV-RPGR vector-treated eyes, but there was no correction of short wavelength (S) opsin mislocalization (data not shown).

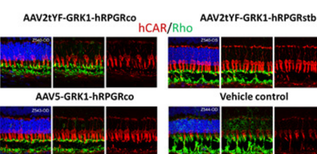


Figure 2. Correction of rod opsin (Rho) mislocalization (in green) in the vector-treated retinal area of eyes receiving AAV-GRK1-RPGR at mid-dose (6×10^{11} vg/mL) compared to the negative control vehicle-treated eye. Cone arrestin labeled with anti-human cone arrestin (hCAR) antibody is in red.

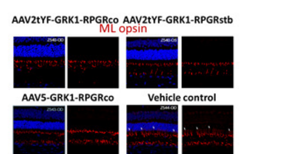
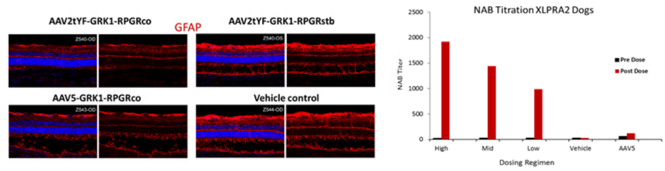


Figure 3. Correction of M/L cone opsin mislocalization (in red) in the vector-treated retinal area of eyes receiving AAV-GRK1-RPGR at mid-dose (6×10^{11} vg/mL) compared to the negative control vehicle-treated eye.

RESULTS (CONTINUED)

IHC staining of Müller cells for glial fibrillary acidic protein (GFAP,) indicative of Müller cell activation, was decreased in eyes injected with the low- and mid-doses (**Figure 4**) but not in the eyes injected with the high-dose. Inflammatory reactions in retinas injected with the high dose (data not shown) may have caused ongoing Müller cell gliosis.



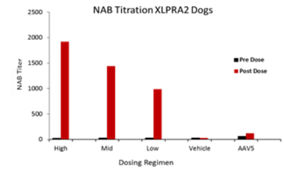


Figure 4 Absent or reduced glial fibrillary acidic protein (GFAP) immunolabeling of Müller cell radial extensions (in red) in some eyes treated with AAV-GRK1-RPGR compared to control vehicle-injected eyes

Figure 5 Average titers of Nab against AAV2tYF or AAV5 determined in dog serum collected at baseline (pre-dose) and at termination (8-weeks post-dose). The PC group received AAV5-GRK1-hRPGRco at mid dose.

All 6 animals that injected with AAV2tYF vectors had increased neutralizing antibody (NAb) titers to AAV2tYF at 8 wks compared to pre-dose titers, with an apparent dose response (**Figure 5**). The magnitude of the NAb response to AAV5 was lower in the animal injected with the AAV5-RPGRco vector at mid-dose (positive control) compared to the titers of NAb to AAV2tYF in animals injected with AAV2tYF-RPGRco at the same dose level.

All serum samples were negative for anti-RPGR antibodies during the study.

CONCLUSIONS

AAV vectors expressing RPGRco or RPGRstb, driven by a GRK1 promoter, packaged in AAV2tYF capsids and delivered by subretinal injection were well tolerated and resulted in RPGR transgene expression in photoreceptors of XLPRA2 dogs treated at mid-stage of disease.

Efficacy was demonstrated with both vectors, including improved structure of CIS (high- and mid-dose), correction of rod and M/L cone opsin mislocalization (all 3 doses), and reduced reactivity of Müller cells (mid -and low-doses).

IHC showed increased RPGR expression at higher doses for both vectors, with the RPGRco construct having substantially higher RPGR expression than the RPGRstb vector at each dose level.

Optimal correction of disease in this model, including improved structure of cone IS, correction of rod and M/L cone opsin mislocalization and reduced reactivity of Müller cells was achieved with the mid-doses of both vector constructs.

REFERENCES

1. Meindl, A., et al., *A gene (RPGR) with homology to the RCC1 guanine nucleotide exchange factor is mutated in X-linked retinitis pigmentosa (RP3).* Nat Genet, 1996. 13(1): p. 35-42.
2. Vervoort, R., et al., *Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa.* Nat Genet, 2000. 25(4): p. 462-6.
3. Beltran, W.A., et al., *Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa.* Proc Natl Acad Sci U S A, 2012. 109(6): p. 2132-7.
4. Beltran, W.A., et al. *Successful arrest of photoreceptor and vision loss expands the therapeutic window of retinal gene therapy to later stages of disease.* Proc Natl Acad Sci U S A, 2015. 112(43): E5844-53
5. Wu, z., et al., *A long-term efficacy study of gene replacement therapy for RPGR-associated retinal degeneration*, HMG, 2015, 24, 3956-3970
6. Ye,G.J. et al., *Cone-specific promoters for gene therapy of achromatopsia and other retinal diseases*, Human Gene Therapy, 2016, 27 (1), 72-82
7. Beltran, W.A., et al., *Optimization of retinal gene therapy for X-linked retinitis pigmentosa due to RPGR mutations*, Mol. Ther., 2017 (in press)

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