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

X-linked retinitis pigmentosa (RP) is a primary rod and cone disease affecting ~20,000 patients in the US and EU.1 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.2,3

The natural history of the RPGR/XLPR2 disease shows that there is a slow decline in ONL thickness in RPGR knockout (KO) mice.4 The RPGR cDNA contains a long purine-rich repetitive sequence in the ORF15 exon that is also seen in the introns.5 The natural history of the RPGR/XLPR2 disease shows that there is a slow decline in ONL thickness in RPGR KO mice.4 The RPGR cDNA contains a long purine-rich repetitive sequence in the ORF15 exon that is also seen in the introns.5 The natural history of the RPGR/XLPR2 disease shows that there is a slow decline in ONL thickness in RPGR KO mice.4 The RPGR cDNA contains a long purine-rich repetitive sequence in the ORF15 exon that is also seen in the introns.5

**RESULTS**

No sustained signs of ocular discomfort or epithelial (including retinal) complications were noted in any of the eyes treated with the mid- or low-doses of AAV2tYF-GRK1-RPGRco or AAV2tYF-GRK1-RPGRstb, AAV5-GRK1-RPGRco (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-RPGRco and AAV2tYF-GRK1-RPGRstb.

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**METHODS**

AAV2tYF-GRK1-RPGRco and AAV2tYF-GRK1-RPGRstb were produced using an HSV-based manufacturing system and purified by column chromatography. A positive control vector, AAV5-GRK1-RPGRco, was produced using a plasmid transfection method and purified by nondenaturing gel electrophoresis. The final concentration of all vectors was in balanced salt solution (BSS) containing 0.14% Tween-20.

Two dogs per group received a 0.15 ml subretinal injection of AAV2tYF-GRK1-RPGRco in the right eye and AAV2tYF-GRK1-RPGRstb in the left eye at each of 3 dose levels (3.2 × 1011, 6 × 1011 or 3 × 1012 vg/mL). One dog received the mid-dose of AAV5-GRK1-RPGRco in both eyes (positive control, NC) and 1pc 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 BSS and enzyme-linked immunosorbent assay (ELISA) and antibodies directed against AAV by a neutralisation assay. Photoreceptor rescue was determined by histology/immunohistochemistry (IHC) analysis of retinal tissues at termination for preservation of retinal structural and functional endpoints of disease, such as opsin miscalocalization and Müller cell gliosis.

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**REFERENCES**


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**RESULTS (CONTINUED)**

**Figure 4** Absent or reduced glial fibrillary acidic protein (GFAP) immunostaining of Müller cell radial extensions (in red) in eyes treated with AAV2tYF-GRK1-RPGR compared to control vehicle-injected eyes. 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-GRK1-RPGRco vector at mid-dose (positive control) compared to the titers of NAb to AAV2tYF vectors at the same dose level.

**Figure 5** Average titer of NAb against AAV2tYF or AAV5 determined in dog serum collected at baseline (pre-dose) and at termination (8 wks post-dose). The PC group received AAV5-GRK1-RPGRco at mid-dose.

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**CONCLUSIONS**

AAV vectors expressing RPGRiso or RPGRshRNA, 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 CS (high- and mid-dose), correction of rod and M/L cone opsin miscalocalization (all doses), and reduced reactivity of Müller cells (mid- and low-dose). HPH showed increased RPHPGR expression at higher doses for both vectors, with the RPGRiso construct having substantially higher RPHPGR expression than the shRNA vector at each dose level. Optimal correction of disease in this model, including improved structure of CS α, correction of rod and M/L cone opsin miscalocalization and reduced reactivity of Müller cells was achieved with the mid-doses of both vector constructs.