Safety and Efficacy of AAV2tYF-GRK1-hRPGRco Vectors in a Canine Model of RPGR-XLRP

GJ Ye[1]; WA Beltran[2]; VL Dufour[2]; AV Cideciyan[2]; LF Marinho[2]; S Iwabe[2]; GS Ying[2]; C Song[1]; PM Robinson[1]; DR Knop[1]; A Timmers[1]; SG Jacobson[2]; GD Aguirre[2]; JD Chulay[1]; MS Shearman[1]

[1]AGTC, Alachua, FL, USA; [2]University of Pennsylvania, Philadelphia, PA, USA

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, 6]

AGTC is developing rAAV2tYF-GRK1-hRPGRco, a recombinant adeno-associated virus expressing full length human RPGR, for treatment of XLRP caused by mutations in the *RPGR* gene. In previous studies we demonstrated that an AGTC proprietary full-length human *RPGR* cDNA (hRPGRco) is stable at each step of molecular cloning and AAV vector production and is effective in functional and structural rescue at early and mid-stage disease in the XLPRA2 dog model of X-linked retinitis pigmentosa caused by an *RPGR* exon ORF15 microdeletion. Here, we report results of a safety and efficacy study of rAAV2tYF-GRK1-hRPGRco (AGTC-501) in RPGR mutant dogs.

METHODS

A total of 16 XLPRA2 dogs (n= 3 to 5 per group, 4 groups) at 5 to 6.5 weeks of age received a subretinal injection in the right eye of 70 µL of vehicle (balanced salt solution with 0.014% Tween 20) or rAAV2tYF-GRK1-hRPGRco vector at a concentration of 1.2×10^{11}, 6×10^{11} or 3×10^{12} vg/mL. The contralateral left eye remained untreated. Safety assessment were conducted during a 20 week post dosage period based on mortality, clinical observations, ophthalmic examinations (tonometry, slit lamp biomicroscopy and indirect ophthalmoscopy), ERG, confocal scanning laser ophthalmoscopy/optical coherence tomography (cSLO/OCT), clinical pathology, and histopathology. Efficacy assessments were based on ERG responses and outer nuclear layer (ONL) thickness analysis (by *in vivo* OCT imaging and histology). Most measurements were performed masked to groups. Immunological analysis included serum antibodies against human RPGR by ELISA and antibodies against AAV2tYF by a neutralization assay. The study design is summarized in **Table 1**.

Table 1 Design of the Safety and Efficacy Study in XLPRA2 Dog

Group	Number of Dogs (Sex)	Vector	Vector Conc. (vg/mL)	Inj Vol (µL)	Dose (vg/eye)	Route
1 (Vehicle)	3 (3M)	Vehicle	0	70	0	Subretinal
2 (Low)	4 (3M/1F)	AAV2tYF-GRK1-hRPGRco	1.2×10^{11}	70	8.4×10^{9}	Subretinal
3 (Mid)	4 (4M)	AAV2tYF-GRK1-hRPGRco	6.0×10^{11}	70	4.2×10^{10}	Subretinal
4 (High)	5 (5M)	AAV2tYF-GRK1-hRPGRco	3.0×10^{12}	70	2.1×10^{11}	Subretinal

RESULTS

No systemic toxicity was associated with treatment during the 20 week study. No significant test article-related effects were observed on body weight, intraocular pressure (IOP), clinical pathology parameters, organ weights, or macroscopic findings. Mild to moderate conjunctival hyperemia and chemosis were found in animals across all groups including the vehicle control group. Ocular histopathology showed no significant histological lesions in any eyes of the mid- and low-dose groups, except for a few no test article-related perivascular episcleral infiltration and/or conjunctival aggregates of lymphocytes and plasma cells noted in 3 eyes (1 untreated, 1 vehicle treated and 1 in mid-dose group). In the eyes treated with the high-dose vector, mild to moderate perivascular infiltrates within the retinal ganglion cell (RGC) layer and optic nerve head were noted (5 of 5 eyes) (**Figure 1, panels A, B, C and D**). Minimum to mild perivascular infiltrates in inner nuclear layer (INL) were also noted in 2 of these 5 eyes (animal ID Z592 OD and Z604 OD), and 1 of these 2 eyes (Z592 OD) had clinical ocular signs of toxicity including chorioretinitis and near to complete loss of photoreceptors in the treated area (**Figure 1**, **panels E & F**). It was noted that minimal perivascular lymphoplasmacytic infiltrates were also observed within the RGC layer of the untreated contralateral eye of this animal (Z592 OS).

RESULTS (CONTINUED)

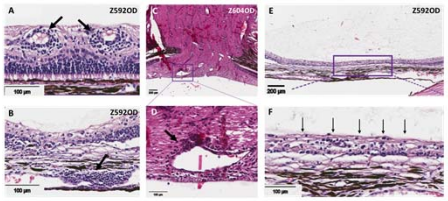

Figure 1 Representative ocular histopathologic images from animals in the high-dose group showing mild to moderate perivascular infiltrates within the retinal ganglion cell layer and inner nuclear layer (**A**), choroid (**B**) and optic nerve head (**C & D**). Near to complete loss of photoreceptors in the treated area in 1 of 5 high dosed eyes was also shown (E & F)

ERG results did not reveal, in any of the treatment groups, any findings that would suggest pan-retinal toxicity. In the animal from the high-dose group (Z592OD) that had severe signs of focal chorioretinitis and retinal atrophy that was limited to the area of treatment, overall ERG function was similar to that of the contralateral uninjected eye.

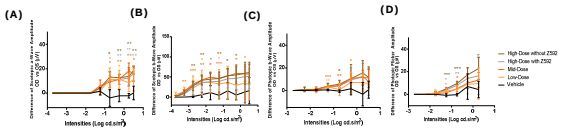

Figure 2 Differences in scotopic a-wave and b-wave amplitudes between injected and uninjected eyes at Weeks 19-20 (A and B). Differences in photopic b-wave and flicker amplitudes between injected and uninjected eyes at 19-20 Weeks (C and D). * = $p \le 0.05$, ** = $p \le 0.01$, *** = $p \le 0.001$

Improved rod, mixed rod-cone, and cone ERG responses were achieved in the eyes of all 3 vector-treated groups (low, mid and high) with statistical significance achieved in the mid-, and high-dose groups when compared to the contralateral untreated eyes, or the vehicle control group (**Figure 2**). Statistically significant preservation of ONL thickness determined by OCT and histology assessment was achieved in eyes of low-, mid-, and high-dose groups (**Figure 3**).

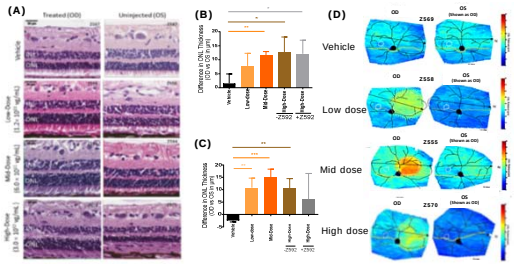

Figure 3 Assessment of ONL retention across treatment groups measured by histology and OCT. (A) Representative photomicrographs of the retinal morphology to compare the ONL thickness of treated (OD) and untreated (OS) eyes. (B and C) Mean (± SD) difference in ONL thickness at Week 10 or Week 19-20 comparing the treated eye (OD) versus untreated contralateral eye (OS) across vehicle, low-, mid-, high- with Z592 and high- without Z592 dose groups. Significance is based on comparison of treatment to vehicle control, with * = $p \le 0.05$, ** = $p \le 0.01$, *** = $p \le 0.001$. (D) Representative topography of ONL thickness in AAV vector-treated (OD) and untreated control eyes (OS) of XLPRA2 dogs shown on a pseudocolor scale with superimposed retinal blood vessels and optic nerve.

RESULTS (CONTINUED)

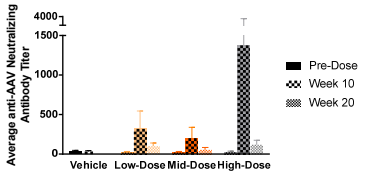

Figure 4 Mean titers of neutralizing antibodies (Nabs) against AAV2tYF measured in dog serum collected at baseline (pre-dose), 10 weeks post-dose and at termination (20 weeks post-dose). Nab titer was averaged from all dogs of each dosing group received rAAV2tYF-GRK1-hRPGRco at low-dose (1.2×10^{11} vg/mL), mid-dose (6.0×10^{11} vg/mL), or high-dose (3.0×10^{12} vg/mL), or the vehicle control (BSS with 0.014% Tween 20).

All 16 animals assigned to the study had detectable low titers of neutralizing antibodies (Nab) to AAV2tYF in the pre-dose phase (mean Nab titer of 22). Nab titers in the vehicle control group remained the same or increased slightly at week 10 or 20. Moderate increase in Nab levels at week 10 was found in all animals in the low- (mean titer: 320) and mid- (mean titer: 200) dose groups and 3 of 5 animals in the high-dose group (mean titer: 160). However, a much higher increase in Nab levels were detected in the remaining 2 of 5 animals in the high-dose group with titers of 1,280 (Animal ID Z592) and 5,120 (Animal ID Z575) at Week 10, which is the cause for the elevated mean Nab level of high-dose group at Week 10 (**Figure 4**). At Week 20, the Nab levels of all animals decreased to levels ranging from 40 to 160.

All samples were negative for anti-hRPGR antibodies at pre-dose, Week 10 and Week 20.

CONCLUSIONS

1. Subretinal injection of AAV2tYF-GRK1-hRPGRco was well tolerated in RPGR mutant dogs at all three doses, with no adverse effects at both the low (1.2×10^{11} vg/mL) and mid (6×10^{11} vg/mL) dose .
2. Evidence of efficacy determined by ERG, OCT and histology were observed with rAAV2tYF-GRK1-hRPGRco delivered subretinally at all 3 doses.
3. Clinical and histological evidence of toxicity was observed at the high dose (3.0×10^{12} vg/mL).
4. Based on these results we conclude the no-observed-adverse-effect-level (NOAEL) of rAAV2tYF-GRK1-hRPGRco injected subretinally in *RPGR* mutant dogs is the 6×10^{11} vg/mL (4.2×10^{10} vg/eye) under the condition of the study.
5. These results support the use of AAV2tYF-GRK1-hRPGRco in clinical studies in XLRP patients caused by mutations in *RPGR*.

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. Beltran, W.A., et al., *Optimization of retinal gene therapy for X-linked retinitis pigmentosa due to RPGR mutations*, Mol. Ther., 2017

Visionary science for life changing cures.