Toxicology and Pharmacology Evaluation of an AAV Vector Expressing Codon-optimized RPGR in RPGR-deficient Rd9 Mice

Chunjuan Song[1], Thomas J Conlon[2], Wen-Tao Deng[3], Kirsten E. Coleman[3], Ping Zhu[3], Peter Sonnentag[4], Alok K. Sharma[4], Adrian Timmers[1], Paulette M. Robinson[1], David R. Knop[1], William W. Hauswirth[3], Jeffrey D. Chulay[1], Mark S. Shearman[1] and Guo-jie Ye[1]

[1]AGTC, Alachua, FL; [2]CR Scientific and Compliance Consulting LLC, Gainesville, FL, [3]University of Florida, Gainesville, FL; [4]Covance Inc, Madison, WI

INTRODUCTION

- X-linked retinitis pigmentosa (XLRP) accounts for approximately 10% of all RP cases, and approximately 80% of XLRP cases are caused by mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene[1]. Analysis of disease progression of patients with XLRP caused by RPGR gene mutations shows a steady deterioration of visual field, development of night blindness, reduced electroretinography (ERG) function at early ages, progressive loss of ERG amplitudes with aging and decline in visual acuity[2,3].
- There are multiple alternatively spliced transcripts of the RPGR gene. An alternatively spliced transcript RPGR-ORF15, is most abundant in the retina in all species examined[4], and localized in the connecting cilia of rod and cone photoreceptors. Some RPGR-ORF15 can also be detected in the outer and inner segments[5,6].
- Proof of concept studies in XLRP mouse and dog models have shown that subretinal delivery of recombinant adeno-associated virus (rAAV) vectors expressing a RPGR-ORF15 can maintain photoreceptor structure and function[7-8].
- AGTC is developing a rAAV vector AGTC-501, also designated AAV2tYF-GRK1-RPGRco, to treat patients with retinitis pigmentosa caused by mutations in RPGR. The vector contains a codon-optimized human RPGR cDNA (RPGRco) driven by a photoreceptor-specific promoter (G protein–coupled receptor kinase 1, GRK1) and is packaged in an AAV2 capsid with three surface tyrosine residues changed to phenylalanine (AAV2tYF). As part of our efforts to develop this vector for use in patients, we conducted a study in the naturally occurring RPGR deficient Rd9 mouse model to evaluate vector safety by standard GLP-compliant toxicology methods, and vector potency by determining dose-related protein expression and localization *in vivo*.

METHODS

- AAV2tYF-GRK1-RPGRco was manufactured using AGTC's proprietary herpes simplex virus (HSV)-based method.
- Sixty Rd9 mice (20 per group; 6-8 weeks of age) received 1 µL subretinal injection in the right eye of vehicle (control) or AAV2tYF-GRK1-RPGRco at one of two dose levels (4×10^8 or 4×10^9 vg/eye). The left eye from all animals was untreated.
- Ten animals per group were sacrificed at 4 weeks and the remaining animals were sacrificed 12 weeks after injection.
- Toxicity assessment was based on mortality, clinical observations, body weight, ophthalmic exams, scotopic and photopic electroretinogram (ERG), organ weight, and clinical and anatomic pathology.
- Potency was assessed by immunohistochemistry (IHC) of RPGR expression in the retina.

Table 1 Study Design

Group	Number of Animals (Sex)	Number of Animals (Sex) at Termination		Vector	Concentration (vg/mL)	Volume (µL)	Total Dose (vg/eye)
		Week 4	Week 12				
1	20 (11M/9F)	10 (6M/4F)	10 (5M/5F)	Vehicle [1]BSS with 0.014% Tween 20)	0	1	0
2	20 (10M/10F)	10 (5M/5F)	10 (5M/5F)	rAAV2tYF-GRK1-RPGRco	4.0×10^{11}	1	4.0×10^8
3	20 (10M/10F)	10 (5M/5F)	10 (5M/5F)	rAAV2tYF-GRK1-RPGRco	4.0×10^{12}	1	4.0×10^9

RESULTS

Mortality and Clinical Observations

- No rAAV2tYF-GRK1-RPGRco-related mortality or clinical signs were observed throughout the study. The subretinal injection of the test article at both dose levels was well tolerated in all groups.

RESULTS (CONTINUED)

Ophthalmic Findings

- No rAAV2tYF-GRK1-RPGRco-related ophthalmic findings were observed at Weeks 4 and 12. All findings (**Table 2**) were considered procedure-related or background findings. Lesions were detected in all eyes receiving subretinal injection for all Groups at Week 4 and Week 12.
- All dosed eyes showed the effect of transcorneal administration procedure. The location of cataract origin in the dosed eyes corresponded with the location of the corneal lesion, consistent with this being an effect of the subretinal injection. Across the treatment Groups, some eyes showed pigment mottling in the fundus in addition to some eyes with retinal hemorrhage or vessel attenuation. These phenomena were also considered a consequence of the surgery.

Table 2 Ophthalmic examination findings for injected eyes

	Group 1: Vehicle		Group 2: Low Dose		Group 3: High Dose	
	Week 4	Week 12	Week 4	Week 12	Week 4	Week 12
Cornea lesion	9/10	10/10	9/9	10/10	10/10	9/9
Cornea opacity	9/10	0/10	6/9	0/10	10/10	0/9
Cornea edema	0/10	1/10	0/9	0/10	0/10	0/9
Synechia	6/10	2/10	2/9	3/10	1/10	1/9
Cataract	10/10	10/10	9/9	10/10	10/10	9/9
Vitreous in anterior chamber	2/10	1/10	1/9	0/10	0/10	1/9
Vitreal degeneration[a]	1/10	2/10	1/9	1/10	0/10	0/9
Mottling pigment	0/10	1/10	0/9	2/10	0/10	4/9
Retinal hemorrhage	0/10	0/10	1/9	0/10	0/10	0/9
Retinal vessel attenuation	0/10	0/10	0/9	1/10	0/10	3/9
Retinal detachment	0/10	0/10	1/9	0/10	0/10	0/9

Immunohistochemistry

- Immunolabeling of RPGR protein, mainly in the inner segment of photoreceptors and the adjacent connecting cilia region, was observed in a dose-dependent manner in all vector-treated eyes examined (**Figure 1**).

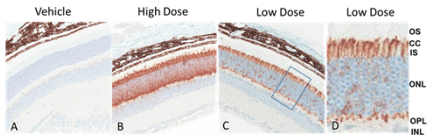

Figure 1 Immunohistochemical staining of RPGR in retinas of mice after subretinal injection of rAAV2tYF-GRK1-RPGRco. Retinal sections of eyes injected with vehicle control (Panel A) or rAAV2tYF-GRK1-hRPGRco at high dose (Panel B) or low dose (Panel C) was stained with anti-human RPGR. The magnified images of the marked areas are shown in (D). Vector-expressed RPGR protein was mainly detected in the inner segment (IS) of photoreceptors and localizes to the connecting cilia (CC) region of the retina. RPGR staining is shown in brown, and nuclei are stained blue by Haematoxylin. OS, outer segments; CC, connecting cilia; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer.

ERG

- Statistical analysis of ERG was performed for the response to scotopic (0.025, 0.25 and 2.5 cds/m^2) and photopic light intensity (1.25, 5, 10 and 25 cds/m^2).
- No statistically significant differences were detected at any flash intensities in the scotopic series nor the photopic series, therefore this difference was considered not to be related to dosing with of rAAV2tYF-GRK1-RPGR.
- Comparison between groups is presented in **Figure 2**, using a representative intensity of scotopic and photopic respectively.

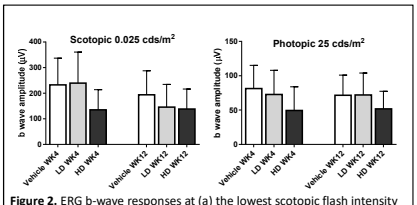

Figure 2. ERG b-wave responses at (a) the lowest scotopic flash intensity (0.025 cds/m^2) and (b) the highest photopic flash intensity (25 cds/m^2).

RESULTS (CONTINUED)

Histopathology

- No rAAV2tYF-GRK-RPGRco-related histopathology findings were observed at Weeks 4 and 12. All findings were considered to be procedure related.
- Retinal findings in the injected eye include the presence of pigmented cells in the subretinal space and within the photoreceptor cell layer, and/or the degeneration of inner and/or outer nuclear layer. In addition, swollen lens fibers and/or lens fibrosis were present in a few animals.
- The findings were present across all groups, including Vehicle controls, and the findings lacked a dose response; as such, these were considered injection procedure related and not related to rAAV2tYF-GRK1-RPGRco (**Table 3** and **Table 4**).

Table 3: Ophthalmic Examination Findings of Injected Eyes (Week 4)

	Group 1: Vehicle		Group 2: Low Dose		Group 3: High Dose	
	Males	Females	Males	Females	Males	Females
Number Examined	6	4	5	5	5	5
Subretinal injection site						
Findings not present	4	1	2	2	1	4
Present	2	3	3	3	4	1
Degeneration, PR/ONL						
Findings not present	5	3	3	3	1	5
Minimal	0	0	1	2	0	0
Slight	0	0	1	0	3	0
Moderate	1	1	0	0	1	0
Pigmented cells, PR, retina						
Findings not present	4	1	2	3	1	4
Minimal	1	2	3	2	1	0
Slight	1	0	0	0	2	0
Moderate	0	1	0	0	1	1
Swollen, lens fibers						
Findings not present	6	3	5	5	5	3
Minimal	0	1	0	0	0	0
Slight	0	0	0	0	0	1
Moderate	0	0	0	0	0	1

Table 4: Ophthalmic Examination Findings of Injected Eyes (Week 12)

	Group 1: Vehicle		Group 2: Low Dose		Group 3: High Dose	
	Males	Females	Males	Females	Males	Females
Number Examined	6	4	5	5	5	5
Subretinal injection site						
Findings not present	4	1	2	2	1	4
Present	2	3	3	3	4	1
Degeneration, INL						
Findings not present	5	3	3	3	1	5
Marked	0	0	1	2	0	0
Degeneration, PR/ONL	0	0	1	0	3	0
Findings not present	1	1	0	0	1	0
Minimal						
Slight	4	1	2	3	1	4
Moderate	1	2	3	2	1	0
Pigmented cells, PR	1	0	0	0	2	0
Findings not present	0	1	0	0	1	1
Minimal						
Slight	6	3	5	5	5	3
Swollen, lens fibers	0	1	0	0	0	0
Findings not present	0	0	0	0	0	1
Minimal	0	0	0	0	0	1
Moderate	0	1	0	0	0	0
Lens fibrosis						
Findings not present	5	4	5	5	5	4
Slight	0	1	0	0	0	0

CONCLUSIONS

- Subretinal injection of AAV2tYF-GRK1-RPGRco in RPGR-deficient Rd9 mice induced predominant RPGR expression in the retina and was well tolerated with no vector-related effects at either 4×10^8 or 4×10^9 vg/eye.
- The no observed adverse effect level (NOAEL) for this study was considered to be 4×10^9 vg/eye.

REFERENCES

1. Fishman GA. Retinitis pigmentosa. Genetic percentages. Arch Ophthalmol. 1978;96(5):822-826.
2. Bird AC. X-linked retinitis pigmentosa. Br J Ophthalmol. 1975;59(4):177-199.
3. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet. 2006;368(9549):1795-1809.
4. Vervoort R, Lennon A, Bird AC, et al. Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. Nat Genet. 2000;25(4):462-466.
5. Khanna H, Hurd TW, Lillo C, et al. RPGR-ORF15, which is mutated in retinitis pigmentosa, associates with SMC1, SMC3, and microtubule transport proteins. J Biol Chem. 2005;280(39):33580-33587.
6. Mavlyutov TA, Zhao H, Ferreira PA. Species-specific subcellular localization of RPGR and RPGRIP isoforms: implications for the phenotypic variability of congenital retinopathies among species. Hum Mol Genet. 2002;11(16):1899-1907.
7. Beltran WA, Cideciyan AV, Boye SE, et al. Optimization of Retinal Gene Therapy for X-Linked Retinitis Pigmentosa Due to RPGR Mutations. Mol Ther. 2017;25(8):1866-1880.
8. Deng WT, Dyka FM, Dinculescu A, et al. Stability and Safety of an AAV Vector for Treating RPGR-ORF15 X-Linked Retinitis Pigmentosa. Hum Gene Ther. 2015;26(9):593-602.

Visionary science for life changing cures.