Safety and Biodistribution Evaluation in Cynomolgus Macaques of rAAV2tYF-PR1.7-hCNGB3, a Recombinant AAV Vector for Treatment of Achromatopsia

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BACKGROUND

AGTC is developing rAAV2tYF-PR1.7-hCNGB3, a recombinant adeno-associated virus (rAAV) vector expressing the human *CNGB3* gene, for treatment of achromatopsia, an inherited retinal disorder characterized by markedly reduced visual acuity, extreme light sensitivity and absence of color discrimination [1]. We report here results of a study evaluating the safety and biodistribution of rAAV2tYF-PR1.7-hCNGB3 in cynomolgus macaques. Results of this study have recently been published [2].

METHODS

- The rAAV2tYF-PR1.7-hCNGB3 vector was manufactured using a recombinant herpes simplex virus (rHSV) complementation system in suspension-cultured baby hamster kidney (sBHK) cells [3], clarified by filtration, purified by affinity chromatography followed by cation-exchange chromatography and formulated in balanced salt solution (BSS) containing 0.014% Tween 20.
- Three groups of animals (n=2 males and 2 females per group) received a subretinal injection in one eye of 300 µL containing either vehicle or rAAV2tYF-PR1.7-hCNGB3 at one of two concentrations (4×10^{11} or 4×10^{12} vg/mL) and were evaluated for safety and biodistribution over a 3-month period prior to euthanasia. Animals were administered test article or vehicle control in the right eye via subretinal injection on Study Day 1 at a volume of 300 mL per eye (2 × 150 µL injections). The left eye was not treated. Study design is shown in **Table 1.**
- Toxicity assessment was based on mortality, clinical observations, body weights, ophthalmic examinations, intraocular pressure (IOP) measurements, electroretinography (ERG), visual evoked potentials (VEP), and clinical and anatomic pathology.
- Vector shedding and biodistribution was assessed by qPCR analyses. Immune responses to AAV and hCNGB3 were measured by ELISA, Elispot, or neutralization antibody assay for AAV2tYF.

Table 1 Study Design

	Number		Dosage Level		
Group	Male	Female	Vector concentration	Injection volume	Total dose
1	2	2	0 (control)	300 µL	0
2	2	2	4.0×10^{11} vg/mL	300 µL	1.2×10^{11} vg
3	2	2	4.0×10^{12} vg/mL	300 µL	1.2×10^{12} vg

RESULTS

- There were no test article-related effects on intraocular pressure, visual evoked potential responses, hematology or clinical chemistry parameters, or gross necropsy observations.
- Biodistribution studies demonstrated that the vector did not spread widely or consistently outside the injected eye. High levels of vector DNA were found in vector-injected eyes but minimal or no vector DNA was found in any other tissue **(Tables 2 & 3)**.
- Serum anti-AAV antibodies developed in all vector-injected animals **(Table 4)**. No animals developed antibodies to CNGB3.
- Histopathological examination demonstrated minimal mononuclear infiltrates in all vector-injected eyes **(Figure 1)**.
- Most manifestations of inflammation improved over time except that vitreous cells persisted in vector-treated eyes until the end of the study. One animal (I05384) in the lower vector dose group was euthanized on Study Day 5 based on a clinical diagnosis of endophthalmitis (**Figure 2**).

RESULTS (CONTINUED)

Table 2 Vector DNA in ocular tissue, visual pathways and cerebellum

Group	1				2				3			
Animal number	I05376	I05377	I05382	I05383	I05378	I05379	I05384	I05385	I05380	I05381	I05386	I05387
Ocular tissue												
Anterior segment (left)	–	–	–	–	–	–	–	–	–	–	–	–
Retina (left)	–	–	–	–	–	–	66	–	–	–	–	–
Optic nerve (left)	–	–	–	–	–	–	3,318	–	–	–	–	–
Anterior segment (right)	–	–	–	–	–	–	17,108	–	–	792	–	–
Retina (right)	–	–	–	–	90,149	141,994	32,778,995	1,512,418	624,453	–	15,146	831,640
Optic nerve (right)	–	–	–	–	–	–	57	–	–	69	–	58
Optic chiasm	–	–	–	–	–	–	–	–	–	–	–	–
Visual pathways												
Optic tract (left)	–	–	–	–	–	–	–	–	–	–	–	–
LGN (left)	–	–	–	–	–	–	–	–	–	–	–	–
Occipital cortex (left)	–	–	–	–	–	–	–	–	–	–	–	–
Optic tract (right)	–	–	–	–	–	–	–	–	–	–	–	–
LGN (right)	–	–	–	–	–	–	–	–	–	–	–	–
Occipital cortex (right)	–	–	–	–	–	–	–	–	–	–	–	–
Cerebellum	–	–	–	–	–	–	–	–	–	–	–	–

Data expressed as vector genome copies (vg) per µg DNA; dash (–) = less than lower limit of quantification (50 vg/ µg of total DNA); LGN = lateral geniculate nucleus
Note: animal I05384 euthanized on Study Day 5, all others on Study Day 92.

Table 3 Vector DNA in lens and vitreous

Group	Animal Number	Copies per sample	Mass per sample (µg)	Copies per µg	Copies per sample	Mass per sample (µg)	Copies per µg
		Lens (left)			Lens (right)		
1	I05376	–	0	NA	–	0	NA
1	I05377	–	0	NA	–	0	NA
1	I05382	–	0	NA	–	0	NA
1	I05383	–	0	NA	–	0	NA
2	I05378	–	0	NA	78	0	NA
2	I05379	–	0	NA	458	0	NA
2	I05384	–	0	NA	60,291	0	NA
2	I05385	–	0	NA	149	0	NA
3	I05380	–	0	NA	332	0	NA
3	I05381	–	0	NA	3,516	0	NA
3	I05386	–	0	NA	494	0	NA
3	I05387	–	0	NA	76,252	0	NA
		Vitreous (left)			Vitreous (right)		
1	I05376	–	0	NA	–	0	NA
1	I05377	–	0	NA	–	0	NA
1	I05382	–	0	NA	–	0	NA
1	I05383	–	0	NA	15	0	NA
2	I05378	–	0	NA	302	0	NA
2	I05379	–	0	NA	13,637	0	NA
2	I05384	–	0	NA	1,008,270	0.02	62,238,893
2	I05385	–	0	NA	14,255	0	NA
3	I05380	–	0	NA	3,713	0	NA
3	I05381	–	0	NA	12,825	0	NA
3	I05386	–	0	NA	–	0	NA
3	I05387	–	0	NA	26,701	0	NA

Data expressed as vector genome copies (vg) per µg DNA; dash (–) = less than lower limit of quantification (50 vg/ µg of total DNA); NA = not applicable
Note: animal I05384 euthanized on Study Day 5, all others on Study Day 92.

Table 4 Serum anti-AAV2tYF antibody titers in cynomolgus macaques

Group	Animal	Sex	Pre-dose	Day 5	Day 92
1	I05376	M	<5		<5
1	I05377	M	<5		<5
1	I05382	F	<5		<5
1	I05383	F	<5		<5
2	I05378	M	10		160
2	I05379	M	<5		80
2	I05384	F	40	20	
2	I05385	F	<5		20
3	I05380	M	10		320
3	I05381	M	<5		160
3	I05386	F	40		320

Animal I05384 euthanized on Study Day 5, all others on Study Day 92.

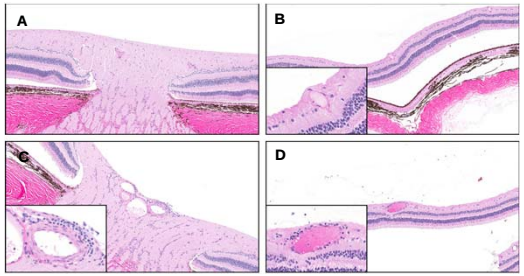

Figure 1. Histological findings. Representative sections showing mononuclear cell infiltrates of minimal intensity in the vitreous body and optic disc in (A) vehicle control eye, (B) normal retina, (C) vector injected eye at dose level 4.0×10^{11} vg/mL, and (D) vector injected eye at dose level 4.0×10^{12} vg/mL.

RESULTS (CONTINUED)

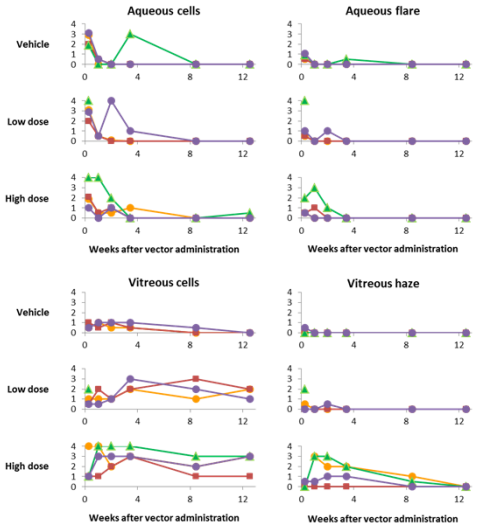

Figure 2. Ocular inflammation findings. Intensity of parameters in individual animals was scored in a standardized fashion as 0, trace (0.5), 1+, 2+, 3+ or 4+. Each color and symbol represents an individual animal, with 4 animals per dosing group.

One animal in group 2 was euthanized at Day 5 based on a clinical diagnosis of endophthalmitis.

One animal in group 1 developed a cataract after an intraoperative posterior lens capsule tear; vitreous cells and haze could not be scored after Study Day 3 in this animal.

CONCLUSIONS

Subretinal injection of rAAV2tYF-PR1.7-hCNGB3 at concentrations of 4×10^{11} or 4×10^{12} vg/mL was associated with a dose-related anterior and posterior segment inflammatory response that improved over time, except that vitreous cells persisted longer than other manifestations of ocular inflammation. There was no evidence of systemic toxicity and no changes in IOP, VEP responses, or hematology, coagulation or clinical chemistry parameters and no clinically important changes in ERG responses. These results support the use of rAAV2tYF-PR1.7-hCNGB3 in clinical studies in patients with achromatopsia. A Phase 1/2 clinical trial evaluating rAAV2tYF-PR1.7-hCNGB3 administered by subretinal injection in patients with achromatopsia is scheduled to begin in 2016.

REFERENCES

1 Kohl S, Jagle H, Sharpe LT and Wissinger B. Achromatopsia (http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=achm). Pagon RA, Bird TC, Dolan CR, Stephens K, editors. Gene Reviews [Internet]. Seattle: University of Washington, 2013.

2 Ye GJ, Budzynski E, Sonnentag P, et al. Safety and biodistribution evaluation in cynomolgus macaques of rAAV2tYF-PR1.7-hCNGB3, a recombinant AAV vector for treatment of achromatopsia. Hum Gene Ther Clin Dev 2016;27:37-48.

3 Thomas DL, Wang L, Niamke J, et al. Scalable recombinant adeno-associated virus production using recombinant herpes simplex virus type 1 coinfection of suspension-adapted mammalian cells. Hum Gene Ther 2009;20:861-870.

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