

Safety and Biodistribution Study of rAAV2tYF-CB-hRS1 in Nonhuman Primates

Guo-jie Ye¹, Ewa Budzynski², Peter Sonnentag², Paul Miller³, Alok K. Sharma², James N. Ver Hoeve³, Kenneth S. Latimer², Kellie Howard⁴, Jeffrey D. Chulay¹

¹AGTC, Alachua, FL; ²Covance Laboratories Inc, Madison, WI; ³OSOD, Madison, WI; ⁴Laboratory Corporation of America® Holdings, Seattle, WA

BACKGROUND

X-linked retinoschisis (XLRs) is an early onset retinal degenerative disease and is the leading cause of juvenile macular degeneration in males. Characteristic features include mild to severe loss in central vision, radial streaks arising from foveal schisis, splitting of inner retinal layers in the peripheral retina, and a negative electroretinogram (ERG) arising from a marked reduction in b-wave amplitude.

AGTC is developing rAAV2tYF-CB-hRS1, a recombinant adeno-associated virus vector expressing retinoschisin (RS1), for treatment of XLRs. Here we report results of a toxicology and biodistribution study of this vector administered by intravitreal injection in cynomolgus macaques.

METHODS

Male cynomolgus macaques received an intravitreal injection in one eye of vehicle or rAAV2tYF-CB-hRS1 at one of two dose levels according to the design in **Table 1**. Half the animals were sacrificed 14 days after vector administration and the others were sacrificed 91 or 115 days after vector administration.

Toxicity assessment was based on mortality, clinical observations, qualitative food consumption, body weights, ophthalmic examinations (slit lamp biomicroscopy and indirect ophthalmoscopy), intraocular pressure measurements (IOP), electroretinography (ERG), visual evoked potentials (VEP, and clinical and anatomic pathology.

Vector biodistribution was assessed by quantitative polymerase chain reaction (qPCR) analyses of blood, urine, feces, nasal secretions, saliva, tears, and ocular, brain, and systemic tissues.

Expression of RS1 protein was assessed based on immunohistochemical analyses of ocular tissues.

Table 1 Design of toxicology study in nonhuman primates

Group	Number	Dosage Level		
		Vector concentration	Volume	Total dose
1	6 males	0 (control)	110 µL	0
2	6 males	3.6×10^{11} vg/mL	110 µL	4×10^{10} vg
3	6 males	3.6×10^{12} vg/mL	110 µL	4×10^{11} vg

RESULTS

There were no test article-related effects on qualitative food consumption, body weights, body weight change, IOP measurements, ERG and VEP measurements, or clinical pathology parameters.

Ocular exams demonstrated a dose-related anterior and posterior segment inflammatory response that improved over time (**Figure 1**). Microscopic pathology results demonstrated minimal to mild mononuclear infiltrates at the optic disc and/or around blood vessels in the ganglion cell layer, iris, and ciliary body of the injected eye in 2 of 6 animals in the low dose group and 4 of 6 animals in the high dose group.

Anti-AAV antibodies were detected in serum from all vector-injected animals at the terminal sacrifice (titer 1:20 to 1:80 at low dose and 1:80 to 1:320 at high dose). No animals developed antibodies to hRS1.

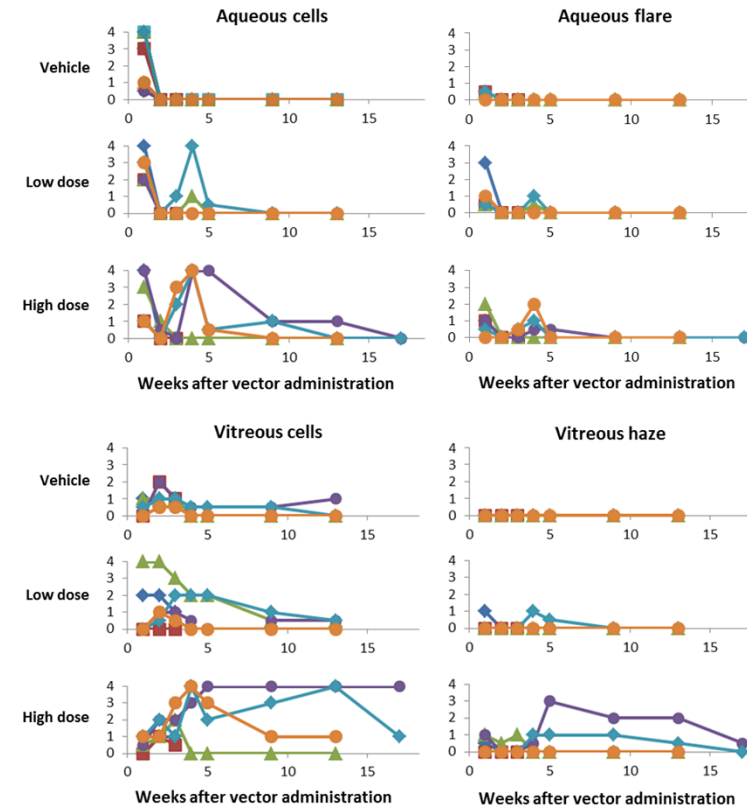


Figure 1 Ocular inflammation findings. Intensity of parameters in individual animals was scored in a standardized fashion as 0, trace (0.5), 1+, 2+, 3+ or 4+.

RESULTS (CONT.)

Immunohistochemical staining showed RS1 labelling of the ganglion cell layer at the foveal slope (**Figure 2**). There was limited vector biodistribution outside the injected eye (**Figure 3**).

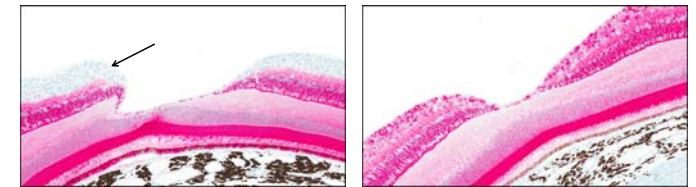


Figure 2 Immunohistochemical staining for RS1. The eye from an animal injected with vehicle control (left) or rAAV2tYF-CB-hRS1 (right) was stained with an antibody specific for human RS1 (red staining). The amino acid sequence of human and cynomolgus RS1 is identical, and the antibody detects RS1 expression in all retinal layers except the ganglion cell layer (blue stained area indicated by an arrow on the left image) in the control eye and also detects RS1 expression in the retinal ganglion cell layer of the vector-injected eye on the right image.

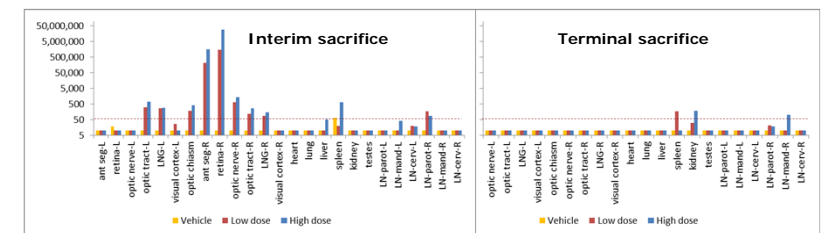


Figure 3 Vector biodistribution. Geometric mean vector copies per µg DNA determined by qPCR. Results below the lower limit of quantification (50 copies per µg DNA) were assigned a value of 10. Anterior segment and retinal tissue at terminal sacrifice were used for histology with no sample for biodistribution.

CONCLUSIONS

Intravitreal administration of rAAV2tYF-CB-hRS1 in normal cynomolgus macaques was associated with dose-related anterior and posterior segment inflammatory response that improved over time. Histological examination showed mononuclear cell infiltrates that were more prevalent at the higher dose level and RS1 expression in the retinal ganglion cell ring was demonstrated by immunohistochemistry. Results from this study support the use of rAAV2tYF-CB-hRS1 in clinical studies in patients with XLRs.



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