Safety and Biodistribution Study of rAAV2tYF-CB-hRS1 in RS1-deficient Mice

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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 RS1-deficient.

METHODS

Male RS1-deficient mice received an intravitreal injection in one eye of vehicle (0.014% Tween 20 in BSS) or rAAV2tYF-CB-hRS1 at one of two dose levels according to the design in **Table 1**. At two time points, 30 days or 90 days after vector administration, half the animals were sacrificed, of which half were used for toxicology and half were used for biodistribution.

In the group scheduled for sacrifice at day 90, an ophthalmic examination was conducted during week 4 and during the week prior to sacrifice. Blood for hematology and clinical chemistry was obtained at sacrifice. Serum for measurement of antibodies to AAV and RS1 was obtained at the 90 day sacrifice. At necropsy, samples of eyes, brain, liver, spleen, heart, lung, kidney, parotid gland and testes were obtained for histopathology and vector biodistribution by qPCR. RS1 expression in ocular tissues was evaluated by immunohistochemistry.

Table 1 Design of toxicology study in RS1-deficient mice

Group	Number	Dosage Level		
		Vector concentration	Volume	Total dose
1	20 males	0 (vehicle control)	1µL	0
2	20 males	1×10^{12} vg/mL	1µL	1×10^{9} vg
3	20 males	4×10^{12} vg/mL	1µL	4×10^{9} vg

RESULTS

The intravitreal injection procedure was well tolerated in all groups. There were no test-article related effects on food consumption, body weight or mortality. Minimally to mildly higher white blood cell and absolute lymphocyte and monocyte counts at Day 90 in two animals in the low dose vector group were suggestive of mild inflammation. There were no other intergroup differences in hematology or clinical chemistry analyses.

Abnormalities on ophthalmic exams were all considered related to the injection procedure or examinations, or congenital or incidental, and were less common at the Day 90 exam than at the Day 30 exam (**Table 2**).

There were no vector-related gross necropsy observations. Microscopic pathology findings were limited to the eye, with minimal to slight mononuclear cell infiltrates in the injected eyes in both vector-treated groups. At the Day 90 sacrifice there was a decrease in the severity of splitting/ disorganization of the inner nuclear layer of the retina in high-dose vector-treated animals. (**Table 3**).

Table 2 Ocular findings at Day 30 and Day 90 (N=10 per group)

	Day 30 examination			Day 90 examination		
	Vehicle	Low dose	High dose	Vehicle	Low dose	High dose
Cataract	7	1	8	3	1	5
Vitreal degeneration	5	3	5	1	1	–
Corneal lesion	4	–	–	2	–	–
Retinal detachment	1	–	–	–	–	–
Retinal opacity	1	–	–	–	–	–
Retinal depigmentation	–	–	–	–	1	–

Table 3 Ocular histopathology findings (N=5 per group)

		Injected eye			Uninjected eye		
		Vehicle	Low dose	High dose	Vehicle	Low dose	High dose
		Interim sacrifice (Day 30)					
Mononuclear cell infiltrate	None	5	–	2	5	5	5
	Minimal	–	5	3	–	–	–
Retinal splitting/ disorganization	Minimal	1	2	2	1	1	1
	Slight	1	–	2	1	–	2
	Moderate	3	3	1	3	4	2
		Terminal sacrifice (Day 90)					
Mononuclear cell infiltrate	None	5	4	4	5	5	5
	Minimal	–	1	–	–	–	–
	Slight	–	–	1	–	–	–
Retinal splitting/ disorganization	Minimal	2	–	5	2	1	4
	Slight	1	2	–	1	1	1
	Moderate	2	3	–	2	3	–

RESULTS (CONT.)

Immunohistochemistry studies showed minimal to moderate RS1 labelling of the retina in the treated eye in both vector-treated groups (**Figure 1**).

At the terminal sacrifice, serum from 9 of 10 vector-treated animals had antibodies to AAV (**Figure 2**). No animal developed antibodies to RS1.

There was no vector biodistribution outside the injected eye (**Figure 3**).

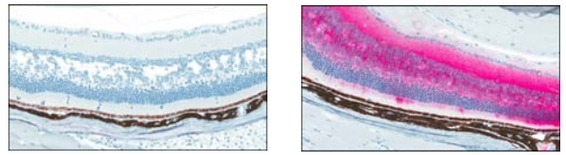

Figure 1 Immunohistochemical staining for RS1. Eyes injected with vehicle (left) or with rAAV2tYF-CB-hRS1 (right) were stained with an antibody specific for human RS1 (red staining).

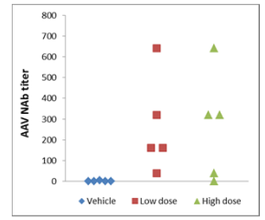

Figure 2 Anti-AAV neutralizing antibody titers. Values are the maximum serum dilution causing ≥50% inhibition of Huh7 cell infection by rAAV2tYF-CMV-lacZ.

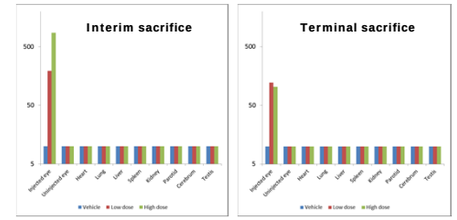

Figure 3 Vector biodistribution. Geometric mean vector copies per µg DNA determined by qPCR. Results below the lower limit of quantification (50 copies per µg DNA) were assigned a value of 10.

CONCLUSIONS

Intravitreal administration of rAAV2tYF-CB-hRS1 in RS1-deficient mice was well tolerated with minimal to slight ocular inflammatory cells detected by histopathology. RS1 expression demonstrated by immunohistochemistry was associated with decreased severity of splitting/disorganization of the inner nuclear layer of the retina at the higher dose level. These results support the use of rAAV2tYF-CB-hRS1 in clinical studies in patients with XLRS.

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