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

Guo-jie Ye¹, Ewa Budzynski², Peter Sonnentag², T. Michael Nork³, Paul E. Miller³, Alok K. Sharma², James N. Ver Hoeve³, Leia M. Smith³, Tara Arndt³, Roberto Calcedo⁵, Chantelle Gaskin¹, Paulette M. Robinson¹, David R. Knop¹, William W. Hauswirth⁶ and Jeffrey D. Chulay¹

AGTC is developing rAAV2tYF-PR1.7-hCNGB3, a recombinant adenovirus-associated virus (rAAV) vector expressing the hCNGB3 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].

RESULTS

The rAAV2tYF-PR1.7-hCNGB3 vector was manufactured using a recombinant herpessimple virus (HSV) complementation system in suspension-cultured baby hamster kidney (BHK) 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 (3 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 × 10¹¹ or 4 × 10¹² 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 µL 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, electrotetroptography (ERG), visual evoked potentials (VEP), and clinical and anatomic pathology.

Vector shedding and biodistribution was assessed by qPCR analysis. Immune responses to AAV and hCNGB3 were measured by ELISA, ELISPOT, or neutralization antibody assay for AAV2tYF.

RESULTS (CONTINUED)

Table 1 Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
<th>Concentration</th>
<th>Total dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0 (control)</td>
<td>300 µL</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4.0 × 10¹¹ vg/mL</td>
<td>300 µL, 1.2 × 10¹² vg</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4.0 × 10¹² vg/mL</td>
<td>300 µL, 1.2 × 10¹³ vg</td>
</tr>
</tbody>
</table>

There were no test article-related effects on intraocular pressure, visual evoked potential responses, hematology or clinical chemistry parameters, or gross necropsy observations.

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

Table 3 Vector DNA in lens and vitreous

Table 4 Serum anti-AAV2tYF antibody titers in cynomolgus macaques

Figure 1. Histological findings.

A B C D

CONCLUSIONS

Subretinal injection of rAAV2tYF-PR1.7-hCNGB3 at concentrations of 4 × 10¹¹ or 4 × 10¹² vg/mL was associated with a dose-related anterior and posterior segment inflammatory response that improved over time, except for 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