Initial Safety Evaluation of rAAV-hCNGB3 Vectors in Nonhuman Primates

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ABSTRACT

- Studies in CNGB3-mutant dogs have shown that subretinal injection of rAAV5-PR2.1-hCNGB3, a rAAV vector expressing the human cyclic nucleotide gated channel beta subunit (hCNGB3) driven by a human red cone opsin promoter (PR2.1) and packaged in AAV5 capsids, can rescue the ACHM ERG phenotype (Komáromy, 2010). However, at high doses many of the animals developed chorioretinitis (Komáromy et al., unpublished observations) and this toxicity (chorioretinitis) is consistent with an immune response to a foreign protein (human CNGB3 has only 76% amino acid identity with canine CNGB3), although toxicity could also be a result of overexpression of CNGB3 protein.
- AGTC is developing a rAAV-hCNGB3 vector for treatment of humans with achromatopsia caused by CNGB3 mutations. Here we report an initial evaluation of the safety in nonhuman primates of rAAV-PR2.1-hCNGB3 vectors packaged in mutant AAV2 (AAV2tYF) or AAV5 capsids. We show that both AAV2tYF-PR2.1-hCNGB3 and AAV5-PR2.1-hCNGB3 were well tolerated after subretinal injection in cynomolgus monkeys at dose levels of 4×10^{10} vg/eye or 4×10^{11} vg/eye. Test article-related findings included dose-dependent ocular inflammation that diminished by Study Week 12, except for presence of white retinal to subretinal foci. No test article-related ERG or cortical visual evoked potential (VEP) effects were observed.
- In contrast to the severe inflammation noted in dogs receiving high dose of AAV5-PR2.1-hCNGB3 that usually resulted in involuntary early termination of the animals, the milder test article-related findings in macaques receiving AAV-PR2.1-hCNGB3 vectors that expresses a highly homologous xenogeneic human protein is helpful for guiding future development of rAAV-CNGB3 gene therapy for human patients.

METHODS

- AAV vectors were manufactured by plasmid transient transfection in HEK 293 cells, purified by double iodixanol step gradient centrifugation, formulated in balanced salt solution (BSS) containing 0.014% Tween 20 and assayed according to the testing requirements defined in the AGTC Standard Operating Procedure (SOP) for rAAV products used in non-GLP animal studies. The quality control testing included assays for identification (for both vector genome and capsid), vector concentration, purity (silver-stained SDS-PAGE0, sterility and endotoxin level. Both rAAV2tYF-PR2.1-hCNGB3 and rAAV5-PR2.1-hCNGB3 passed all release testing.
- Eight cynomolgus macaques were assigned to four groups and received bilateral subretinal injections of AAV2tYF-PR2.1-hCNGB3 or AAV5-PR2.1-hCNGB3, as indicated in **Table 1**. Dose formulations were administered at a volume of 140 µL/eye using the DORC™ dosing apparatus following a study-specific procedure. Animals were observed for 3 months following dose administration. Ophthalmic examinations, digital fundus photography, scotopic and photopic electroretinography (ERG) and visual evoked potentials (VEP), and histology were used to evaluate ocular tolerability of test article.

Table 1 Study design in non-human primates

Group	Number of animals	Vector	Volume injected	Conc. (vg/mL)	Dose (vg/eye)
1 (Low)	2	AAV5-PR2.1-hCNGB3	140 µL	2.88×10^{11}	4×10^{10}
2 (Low)	2	AAV2tYF-PR2.1-hCNGB3	140 µL	2.88×10^{11}	4×10^{10}
3 (High)	2	AAV5-PR2.1-hCNGB3	140 µL	2.88×10^{12}	4×10^{11}
4 (High)	2	AAV2tYF-PR2.1-hCNGB3	140 µL	2.88×10^{12}	4×10^{11}

RESULTS

- In a previous study, in which CNGB3 mutant dogs were injected subretinally with AAV5-PR2.1-hCNGB3, in-life clinical ophthalmic examinations observed retinal thinning and multifocal chorioretinitis characterized by aggregations of inflammatory cells (black spots) at the 5×10^{12} vg/mL dose (**Figure 1B**), but not in any eyes at the dosage level of 5×10^{11} vg/mL (**Figure 1A**). The multifocal chorioretinitis was first visible 5 weeks after treatment and then became more severe thereafter if not medically treated.
- Histological evaluation of the same eye as (**B**) showed that the black spots represent focal accumulations of inflammatory cells in the subretinal space near the outer nuclear layer (ONL) (**Figure 1C**). The ONL containing the photoreceptor cell nuclei appeared disrupted, and the photoreceptor outer and inner segments were destroyed and no longer visible.

Figure 1 Representative photographs from in vivo ocular examination and retinal histopathology of canine eyes injected with rAAV5-PR2.1-hCNGB3. (A) Retina after injection with rAAV5-PR2.1-hCNGB3 at a concentration of 5×10^{11} vg/mL. Two procedure-related black scars are indexed by arrows. (B) Retina after injection of the same vector at a higher concentration of 5×10^{12} vg/mL. (C) Histological evaluation of the same eye as (B). Focal accumulations of inflammatory cells in the subretinal space near the outer nuclear layer (ONL) are indexed by arrow and stained in purple.

- In the present study, in which cynomolgus macaques were injected subretinally with AAV5-PR2.1-hCNGB3 or AAV2-PR2.1-hCNGB3, test article-related ophthalmic findings included usually mild (Trace to 1+) to moderate (2+) but occasionally moderately severe (3+) to severe (4+) anterior and posterior segment inflammatory response that resolved without sequelae (**Figure 3**). In addition, white retinal to subretinal foci within the dose site were observed at Study Week 12 in all four groups (**Figure 2**). The inflammatory response tended to be less intense in eyes given rAAV5-PR2.1-hCNGB3 vs. rAAV2tYF-PR2.1-hCNGB3 and at the lower dose (4×10^{10} vg/eye vs. 4×10^{11} vg/eye). No test article-related ERG and cortical visual evoked potential (VEP) effects were noted.
- Microscopic findings associated with injection of the test articles included minimal retinal degeneration, hypertrophy of retinal pigment epithelium, and/or mononuclear cell infiltrates of the choroid and/or retina. Mononuclear cell infiltrates generally correlated with the subretinal foci noted ophthalmoscopically (**Figure 2**).

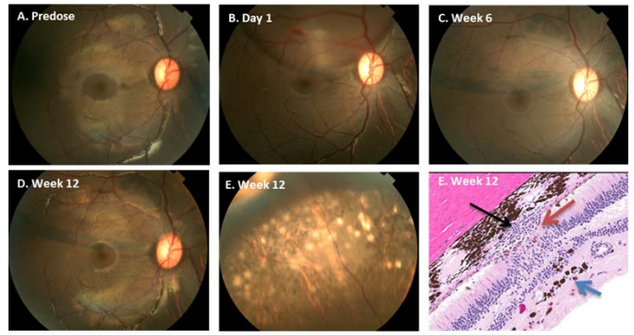

Figure 2 Representative photographs from in vivo ocular examination and retinal histopathology of NHP eyes injected with rAAV5-PR2.1-hCNGB3. (A-E) Retina before and after injection with rAAV5-PR2.1-hCNGB3 at a concentration of 4×10^{11} vg/mL. (E) White retinal to subretinal foci within the dose site first observed at 12 weeks after injection, suggesting a localized inflammatory response. (F) Histological evaluation of the same eye showed mononuclear cell infiltrates in the choroid and retina (black arrow), degeneration of the retina (blue arrow), and hypertrophy of the retinal pigment epithelium (red arrow).

RESULTS (CONTINUED)

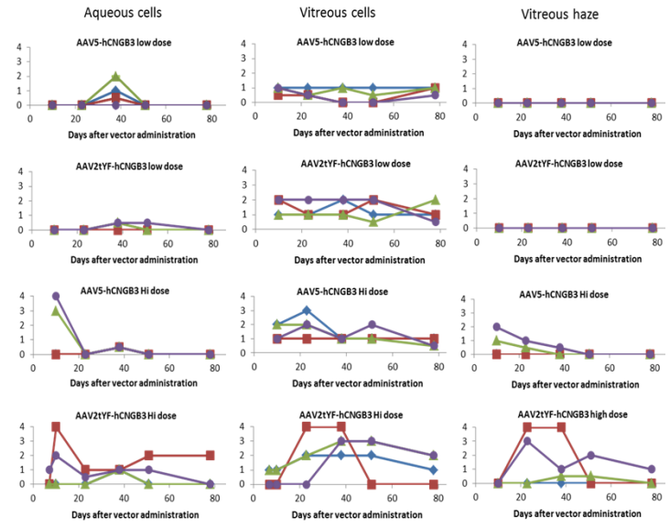

Figure 3 Ocular inflammation findings. Cells were graded as 0 (no cells), 0.5 (1 – 5 cells) , 1 (6-25 cells), 2 (26-50 cells), 3 (51-100 cells), and 4 (> 100 cells) for each eye of each group using a method slightly modified from the Standardization of Uveitis Nomenclature (SUN) method. Vitreous haze were graded according to the SUN method as 0 (no haze), 0.5 (slight blurring of optic disc margin and normal striations and reflex of nerve fiber layer cannot be visualized), 1 (Mild haze with better definition of optic nerve head and retinal vessels than with grade 2), 2 (Moderate haze with better visualization of retinal vessels than with grade 3), 3 (Optic nerve head visualized but borders are quite blurry), 4 (Optic nerve head is obscured).

CONCLUSIONS

- Both AAV2tYF-hCNGB3 and AAV5-hCNGB3 were well tolerated after subretinal injection in NHPs at a dose level of 4×10^{10} or 4×10^{11} vg/eye.
- Test article related findings included dose-dependent ocular inflammation that diminished by Study Week 12. No test article-related ERG or cortical visual evoked potential (VEP) effects were observed.
- In contrast to the severe inflammation noted in dogs receiving high dose of AAV5-hCNGB3 that usually resulted in involuntary early termination of the animals, the milder test article-related findings in macaques receiving AAV-hCNGB3 vectors that expresses a highly homologous xenogeneic human protein is helpful for guiding future development of rAAV-CNGB3 gene therapy for human patients.

REFERENCES

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