# #457 Ocular Toxicity and Efficacy of rAAV2tYF-PR1.7-hCNGB3 Vector Following Subretinal Injection in a Mouse Model of Achromatopsia

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## Introduction

Mutations in the cone-specific cyclic nucleotide-gated channel beta subunit (CNGB3) gene account for about 50% of cases of achromatopsia, an autosomal recessive retinal disease affecting functionality of cone photoreceptors[1]. The phenotype of CNGB3$^{-/-}$ mice is similar to that in humans, specifically loss of cone function and early onset, slowly progressive cone degeneration, making it an appropriate disease model to study both toxicity and efficacy of the treatment[2,3].

### Objective

The purpose of this study was to evaluate ocular tolerability and efficacy in rescuing cone phenotype of a recombinant adeno-associated viral (rAAV) vector expressing human CNGB3 (hCNGB3) under control of a PR1.7 cone-specific promoter (rAAV2tYF-PR1.7-hCNGB3) over 3 months.

## Methods

### Study Design

CNGB3 knockout or C57B6 wild type mice were used. Single subretinal injections (1.1 µL) of either rAAV2tYF-PR1.7-hCNGB3 or a balanced salt solution (BSS) containing 0.014% Tween 20 (Vehicle) were given on Day 1. Formulations contained 0.1% fluorescein to aid in visualizing dose deposition. Left eyes were not treated.

| # Male | Strain | Treatment OD | Dose Level (vg/eye) | Treatment OS |
|---|---|---|---|---|
| 12 | CNGB3 Knockout | Vehicle | 0 | Untreated |
| 12 | CNGB3 Knockout | rAAV2tYF-PR1.7-hCNGB3 | 1 x $10^9$ | Untreated |
| 12 | CNGB3 Knockout | rAAV2tYF-PR1.7-hCNGB3 | 4.7 x $10^9$ | Untreated |
| 12 | Wild Type | Vehicle | 0 | Untreated |

vg - vector genomes

General health was monitored daily for clinical evidence of toxicity. Body weight and food consumption were recorded weekly.

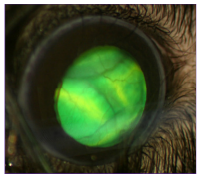
Slit lamp biomicroscopy, indirect ophthalmoscopy, scotopic and photopic electroretinography (ERG) luminance-response series, and light-adapted flicker ERG were performed monthly for assessment of ocular tolerability and cone phenotype rescue.

Eyes were collected and examined by light microscopy.

*Presented at SOT 2015*

### Subretinal Injection

Mice were anesthetized. Following mydriasis, the cornea was incised using a needle or blade. A blunt-tipped needle (31-33 ga) was inserted through the corneal incision toward the back of the eye while avoiding the lens. The dose was injected into the subretinal space using a 5-µL or 10-µL Hamilton syringe causing a focal retinal detachment.



Subretinal deposition of dosing solution containing fluorescein.

## Results

### General Health

There were no clinical observations, or changes in food consumption or body weight attributable to rAAV2tYF-PR1.7-hCNGB3

### Clinical Ophthalmic Exam

There were no clear or consistent findings considered related to rAAV2tYF-PR1.7-hCNGB3.

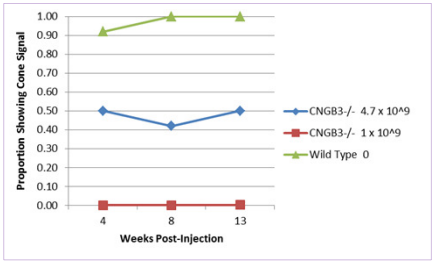
Observations attributed to the animal model or injection procedure limited to some extent the evaluation and included the following:

- Incomplete pupil dilation attributed to the animal model
- Corneal edema, fibrin and scars at the injection site, anterior chamber inflammation, cataract (generally punctate or incipient), retinal and vitreous hemorrhage, and dose site pigment changes.

Findings were related to the transcorneal injection and, except for corneal scars and cataracts, most resolved.

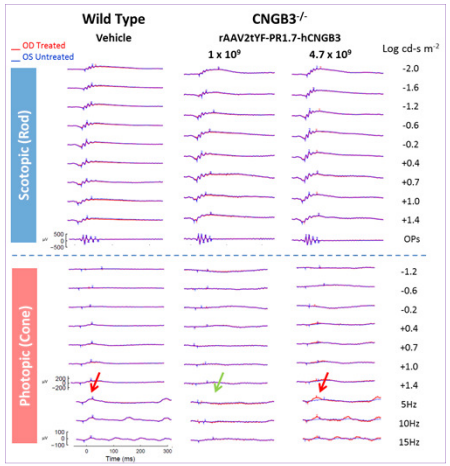
### Electroretinography (ERG)

ERG traces were evaluated qualitatively (present or absent) for the presence of a cone signal. A cone signal was clearly present in wild type mice to the brightest photopic single flash and most clearly to the flicker stimuli.



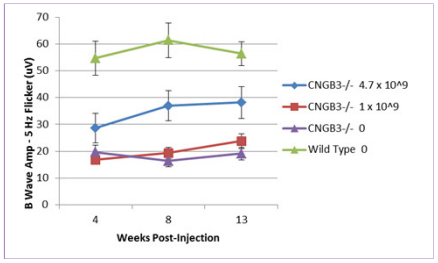

Incidence of cone ERG response in right (treated) eyes at 4, 8 and 13 weeks.

### Electroretinography



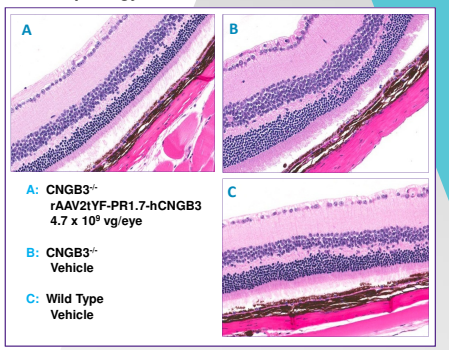

Example scotopic and photopic ERG recorded during Week 8 from treated right eyes (red trace) and untreated left eyes (blue trace).

Note normal rod response present in treated and untreated eyes of CNGB3$^{-/-}$ mouse. Cone response (red arrow) is present in wild type eyes and in the right CNGB3$^{-/-}$ eye given 4.7 x$10^9$ vg/eye rAAV2tYF-PR1.7-hCNGB3, but absent in the mouse receiving the 1.0 x$10^9$ vg/eye dose (green arrow).




Group mean B wave amplitude from 5 Hz photopic white flicker in right (treated) eyes at 4, 8 and 13 weeks. Restored cone signal was evident at 4.7 x$10^9$ vg/eye rAAV2tYF-PR1.7-hCNGB3. Data are mean ± SE (n= 11 or 12).

### Histomorphology




No morphological differences were apparent between wild-type and CNGB3$^{-/-}$ retinas. There were no rAAV2tYF-PR1.7-hCNGB3-related findings noted. Pigmented cells in the retina/subretinal space, retinal degeneration, and/or degeneration/fibrosis of lens were observed at a minimal severity in the right eye of several animals across the treatment groups and were considered procedure-related. Separation between photoreceptor and retinal pigment epithelium layers is artifact.

## Summary

- rAAV2tYF-PR1.7-hCNGB3 was well tolerated as assessed by ophthalmic examinations and not associated with microscopic histomorphologic changes through 3 months after subretinal delivery.
- Starting at 1 month post-dose administration, rAAV2tYF-PR1.7-hCNGB3-treated eyes showed an increase in single flash photopic ERG and flicker sensitivity compared with vehicle-treated eyes, indicating restoration of cone function at 4.7 X $10^9$ vg/eye. No adverse effects were noted on rod function.
- These results support further development of rAAV2tYF-PR1.7-hCNGB3 as a potential treatment for patients with achromatopsia.

## References

1. Kohl S, *et al.*, CNGB3 mutations account for 50% of all cases with autosomal recessive achromatopsia. Eur J Hum Genet 2005;13:302-8.
2. Komaromy AM, *et al.*, Gene therapy rescues cone function in congenital achromatopsia. Hum Mol Genet 2010;19:2581-93.
3. Carvalho LS, *et al.*, Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. Hum Mol Genet 2011;20:3161-75.

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