Proof-of-Concept Studies in Mongolian Gerbils Support Intravitreal Gene Replacement Therapy of Human L-Opsin for Blue Cone Monochromacy

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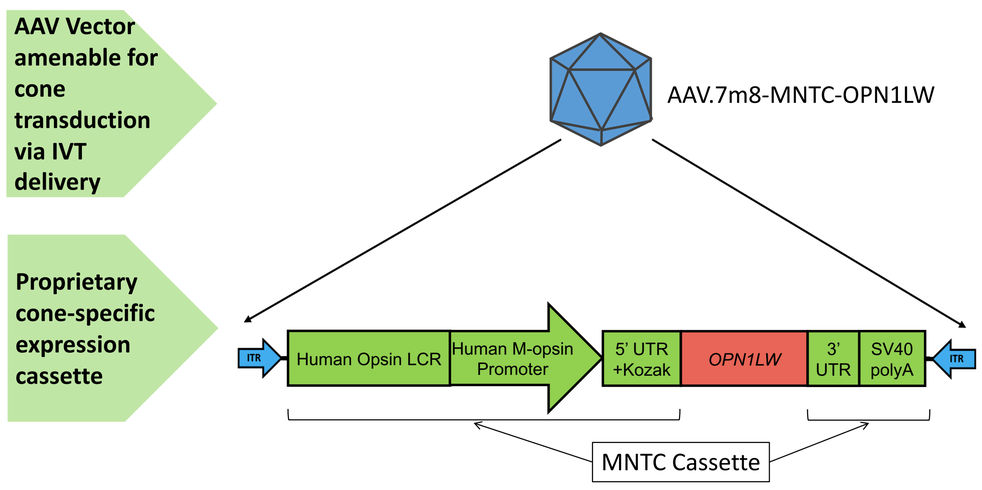
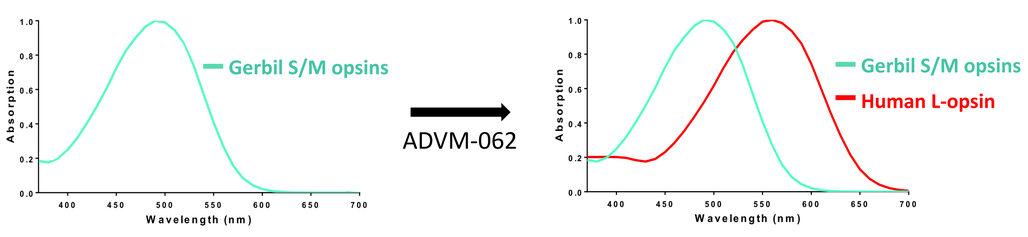
Background and Purpose

- Blue cone monochromacy (BCM) is a rare X-linked visual disorder caused by the genetic deficiency of both the long-wave (L) and medium-wave (M) opsin pigments in cone photoreceptor cells in the retina.
- BCM causes severe color vision deficiency, poor visual acuity, nystagmus and photophobia, and has a high unmet medical need for treatment.
- Intravitreal (IVT) AAV-mediated gene therapy is one of the most promising and minimally invasive approaches to treat retinal diseases such as BCM.
- Adverum is developing ADVM-062 (AAV.7m8-MNTC-OPN1LW) for cone-specific expression of human L-opsin, optimized for IVT delivery, as a potential gene therapy treatment for BCM.
- ADVM-062 is an AAV.7m8 capsid vector containing human L-Opsin cDNA under the control of the MNTC cassette.
- The MNTC expression cassette was engineered to drive efficient and specific gene expression in cone photoreceptors.
- The AAV.7m8 variant efficiently transduces retinal cells, including foveal photoreceptors, from IVT administration. AAV.7m8-based vectors are currently being evaluated in two clinical trials in neovascular AMD and Retinitis Pigmentosa (ClinicalTrials.gov ID: NCT03748784, NCT03326336).
- We previously reported long-term ADVM-062 activity to red light stimuli (630 and 660 nm) by electroretinography (ERG) at a single time point of 86 weeks post-dose in cone-rich Mongolian gerbils (*Meriones unguiculatus*), which naturally lack L-opsin.
- In the current study, we further evaluated ADVM-062 by assessing its dose response, the time course of its functional activity, as well as the localization of L-opsin protein in cones using a c-myc-tagged protein.

Components of the ADVM-062 gene therapy vector



Hypothetical Absorption Spectra of Cones in ADVM-062 Treated Gerbil



Study Design and Methods

- 13-week-old Mongolian gerbils were IVT injected bilaterally with ADVM-062 at 3.9x10^8, 3.9x10^9, 3.9x10^{10}vg/eye (n=5/group), 3.9x10^{11}vg/eye (n=11), or with ADVM-062.myc at 3x10^{11}vg/eye (n=6) and vehicle (n=9).
- Functional expression of human L-opsin was assessed by full-field color ERG responses to red light stimuli over a period of 22 weeks post-dose.
- All ERGs were recorded following adaptation to a rod-desensitizing green background light at 530 nm. Activity mediated by the human L-opsin transgene was assessed using 630 and 660 nm (red and near far-red, respectively) light flashes. ERGs were recorded in response to a series of increasing flash intensities and to flicker frequencies of 25 Hz to isolate cone responses.
- Immunofluorescence staining of eye tissues dosed with ADVM-062.myc was performed using antibodies against myc-tagged L-opsin in a subset of animals at 8 weeks post-treatment.

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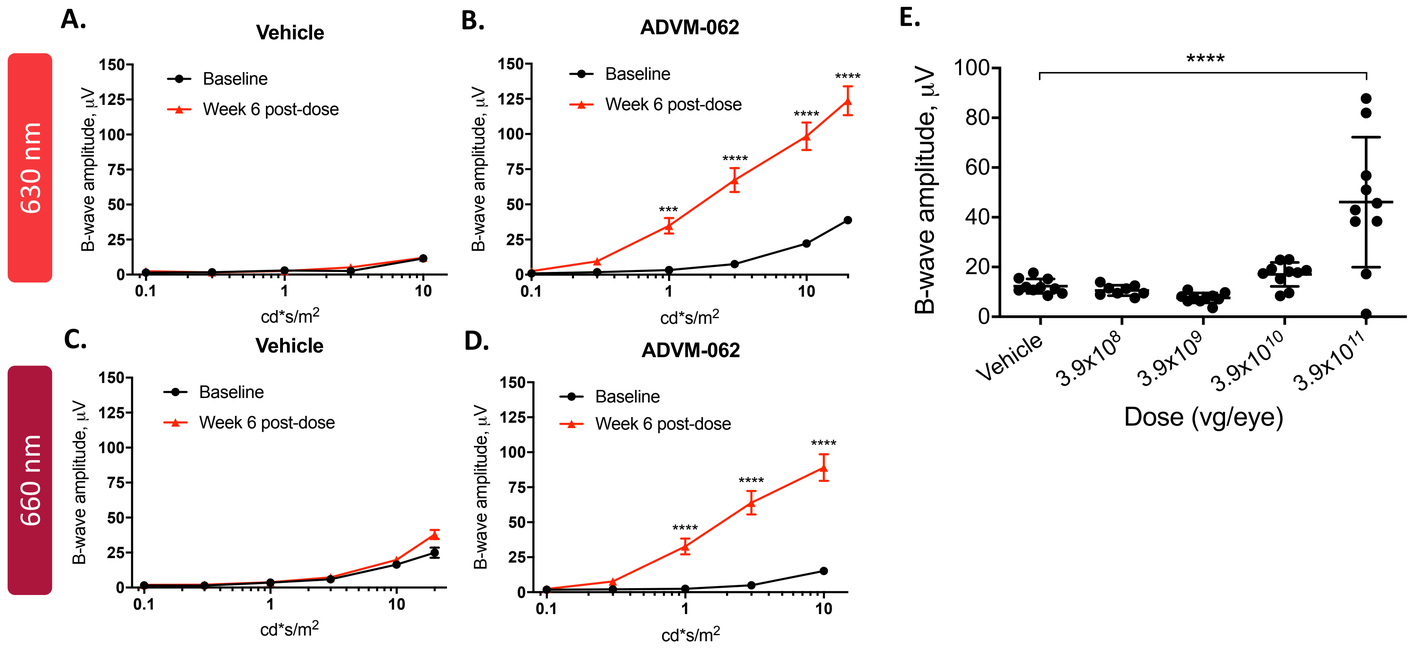
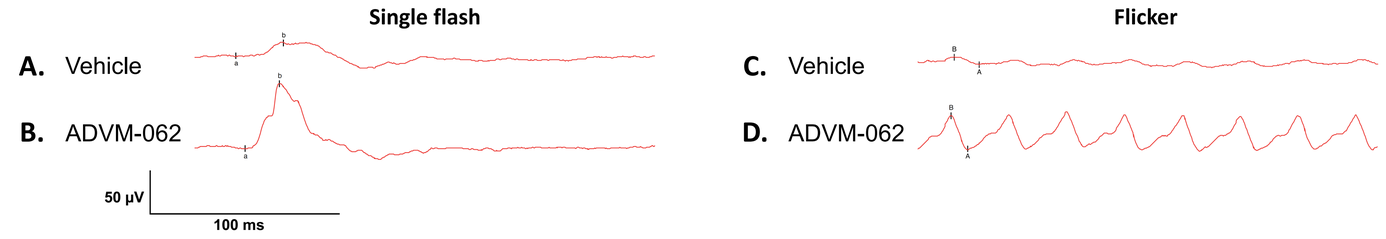
Results

Single IVT dose of ADVM-062 significantly increases gerbil retina sensitivity to red and near-far-red light intensities in a dose-dependent manner



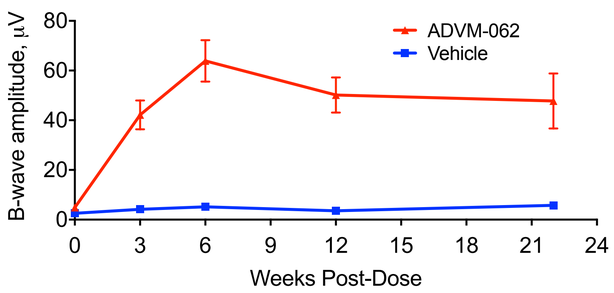
A-D. Color ERG b-wave amplitudes elicited by short flashes of 630 nm (red, *A, B*) and 660 nm (near far-red, *C, D*) lights, respectively, at increasing intensities in the presence of Ganzfeld background of 513 nm. Recordings were performed at baseline and 6 weeks post-dose in vehicle *(A, C)* and ADVM-062-treated gerbils at 3.9x10^{11}vg/eye *(B, D)*. Error bars: SEM, Significance ***: P<0.001; ****: P<0.0001 (RM 2-way ANOVA with Sidak's multiple comparisons test). **E.** ERG responses to 660 nm light stimuli of fixed intensity (3 cd.s/m^2). Recordings were performed 12 weeks post-dose in vehicle control and ADVM-062-treated gerbils at indicated doses. Error bars: SEM, Significance ****: P<0.0001 (One-way ANOVA with Dunnett's multiple comparisons test).

ADVM-062 strongly augments ERG responses to red light stimulus at 25 Hz cone-isolating flicker



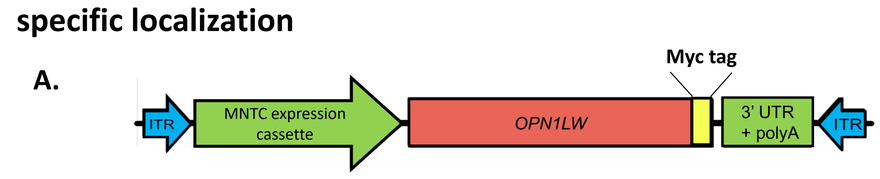
Representative ERG traces of vehicle treated *(A, C)* and ADVM-062-treated gerbils at 3.9x10^{11}vg/eye *(B, D)* at 5 weeks post-dose. ERG responses were elicited by short flashes of 660 nm light at fixed intensity of 10 cd.s/m^2 and frequencies of 1 Hz *(A, B)* or 25 Hz flicker *(C, D)*.

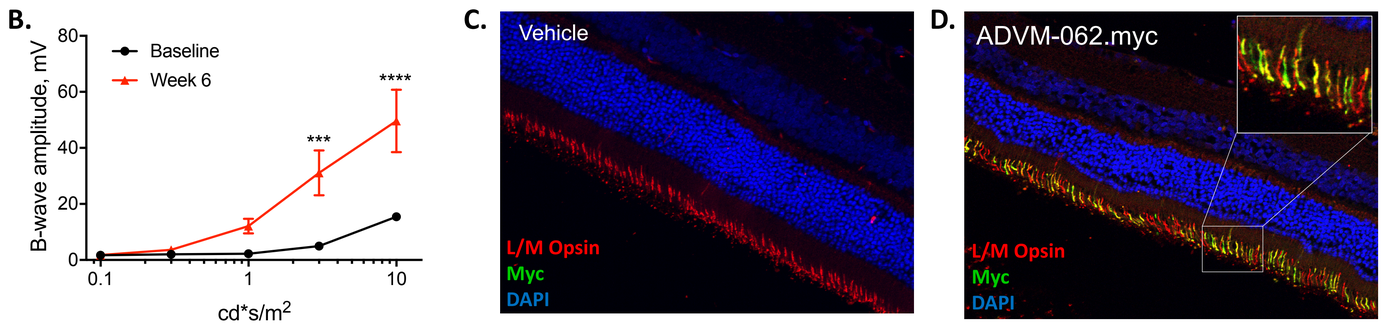
ADVM-062-mediated sensitivity to red light is observed at the first time point evaluated, 3 weeks post-dose



Durability of augmented sensitivity to 660 nm light at fixed 3cd.s/m^2 intensity, shown as b-wave amplitude, in vehicle and ADVM-062-treated gerbils (3.9x10^{11}vg/eye) at baseline, 3-, 6-, 12- and 22-weeks post-dose. Error bars: SEM.

Myc-tagged human L-opsin similarly augments red-light sensitivity in retina and displays cone-specific localization





A. Diagram of the ADVM-062.myc expression cassette encoding C-terminal myc-tagged human L-opsin. **B.** ERG b-wave amplitudes in response to red (660 nm) light stimulus of increasing intensity at baseline and 6 weeks post-IVT dose of 3×10^{11}vg/eye ADVM-062.myc. **C, D**. Immunofluorescence labeling of gerbil retinal tissue sections 8 weeks post IVT dosing with *(C)* vehicle or *(D)* ADVM-062.myc. Co-labeling of myc-tag (green) and L/M-opsin (red) confirms ADVM-062.myc expression in cone photoreceptors *(yellow signal in boxed insert in D).*

Conclusions

- ADVM-062 drives **effective** and **specific expression in cones** to sensitize them to long wavelength stimuli **as early as 3 weeks** following a **single intravitreal** injection in Mongolian gerbil eyes.
- These findings further support the development of ADVM-062 as a potential IVT-delivered treatment for BCM.