Long-term functional delivery of the human L-opsin cDNA via intravitreal administration of an AAV vector in Mongolian gerbils

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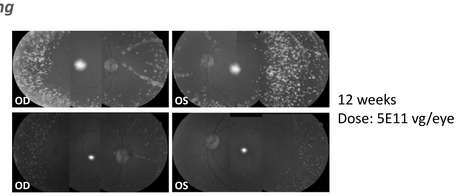
Background

- Blue cone monochromacy (BCM) is a rare X-linked visual disorder that affects of 1/100,000 individuals, with a high unmet medical need for treatment.
- BCM causes severe color vision deficiency, poor visual acuity, nystagmus and photophobia, resulting from the deficiency in the long-wave (L) and medium-wave (M) opsin pigments in cone photoreceptor cells in the retina.
- AAV-mediated gene therapy using intravitreal (IVT) administration is one of the most promising and minimally invasive delivery systems to treat retinal diseases.
- AAV.7m8 is a variant of AAV2 discovered by directed evolution in the retina. It contains a 10 amino acid insertion in Loop IV of AAV2 VP3 (Dalkara *et al*, 2013).
- AAV.7m8 efficiently transduces photoreceptors when delivered by IVT injection.
- The regulatory cassette MNTC was engineered to drive efficient and specific gene expression in L- and M-cone photoreceptors.
- MNTC confers more pronounced and abundant reporter expression in cones in nonhuman primate retina transduced by IVT injection, as compared to the well-characterized cone-specific PR2.1 promoter.

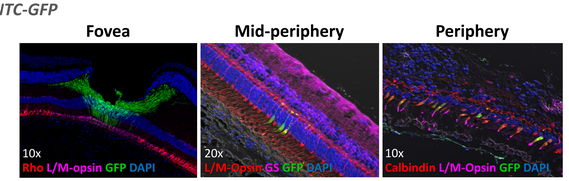
Robust Cone-Specific Expression Mediated by IVT Administration of AAV.7m8-MNTC-GFP in Nonhuman Primate Retina

MNTC Cassette

In vivo Fundus Imaging



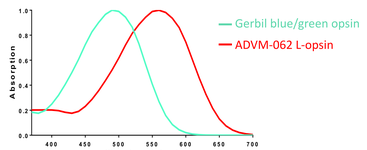
Immunofluorescence staining of NHP retina tissue transduced with AAV.7m8-MNTC-GFP



Purpose

- Adverum is developing ADVM-062 as an intravitreal gene therapy vector to treat blue cone monochromacy.
- ADVM-062 is an AAV.7m8 capsid vector containing human L-opsin (OPN1LW) cDNA under the control of the pMNTC cassette to drive cone-specific gene expression.
- The aim of the study was to assess whether IVT delivery of AAV.7m8-MTNC-LOpsin gene (γ_{peak} = 560 nm) in the Mongolian gerbil could translate into a functional ERG readout.

Hypothetical ADVM-062 Treated Gerbil



Study Design and Methods

- Mongolian gerbils were used as a model for BCM in that their retina contains 11-14% cone cells with peak light absorbance in the short and medium wave (UV-blue/green) spectral regions. Gerbils lack long-wave (red) sensitive cone visual pigment.
- Animals were injected IVT with a single 3E11 vg/5 µl dose of ADVM-062, or formulation buffer in both eyes (n=4 animals).
- Ocular examinations were performed before dosing and throughout the study.
- Functional expression of human L-opsin was evaluated using full-field color electroretinogram (cERG) with light emitting diodes of different wavelengths, at different intensities and frequencies, for over 1.5 years. Exposure to green light prior to and during cERG measurements was used to reduce M-cone contribution to long wavelength light responses.

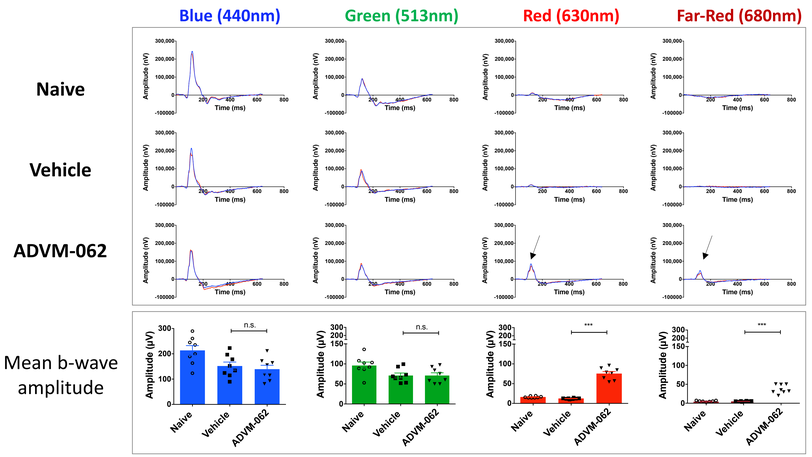
cERG Parameters	Blue (440nm)	Green (513 nm)	Red (630 nm)	Far Red (680 nm)
Flash Intensity	0.1 cd.s/m^2	0.5 cd.s/m^2	3.0 cd.s/m^2	1.0 cd.s/m^2
Flash Frequency	1 Hz	1 Hz	1 Hz	1 Hz
Green Background	30 cd.s/m^2	30 cd.s/m^2	30 cd.s/m^2	30 cd.s/m^2

- Histopathology, immunofluorescence staining and mRNA expression were performed on eye tissues and brain at termination, 90 weeks post-treatment.

Results & Conclusion

- IVT injection of ADVM-062 was well tolerated based on bright field ocular exams and histopathology of ocular tissue.
- Increased cERG responses to red and far-red light were observed from **week 14** and up to **week 90** following ADVM-062 IVT administration.

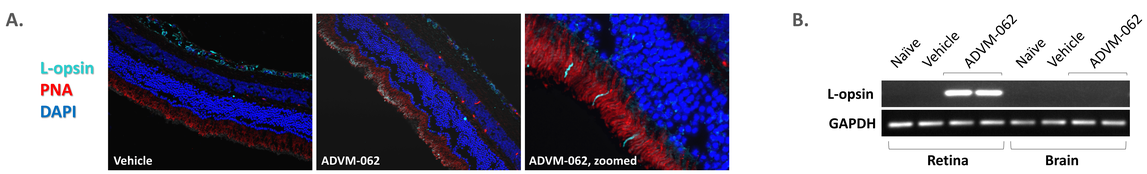
cERG Recordings in Green-Light Adapted Gerbil Eyes



Top: *Representative cERGs tracings of naïve control, vehicle-treated and ADVM-062-dosed gerbils, at 86 weeks post-dosing. Arrows point to the cone-driven b-wave elicited by red and far-red light sources observed in ADVM-062-treated gerbils, which is not observed in control eyes.*

Bottom: *Scatter and bar plots of the mean b-wave amplitude from a total of 8 eyes per group. Error bars: SEM. Significance ***: p<0.001 (non-parametric Wilcoxon-Mann-Whitney test).*

L-Opsin protein is expressed in cone cells of retinas treated with ADVM-062, and is not detected in brain tissue



A. *Immunofluorescence labeling of gerbil retinal tissue sections 90 weeks post IVT dosing. Co-labeling of L-opsin with peanut agglutinin lectin (PNA) confirms ADVM-062 expression in cone photoreceptors.* ***B.*** *RT-PCR of human L-opsin and gerbil GAPDH in retina and brain samples.*

Conclusion and Future Directions

- Our data indicates that ADVM-062 produces **functional, long-term** L-opsin expression following single **intravitreal** delivery in Mongolian gerbil eyes.
- Future validation studies in a mouse model of BCM will be performed to investigate the functional state of photoreceptors that do not express opsin.
- Electrophysiology and behavioral studies in non-human primates will aim at determining if the ERG signal elicited by the long-wave light sources is transmitted to the visual cortex.

Disclosures

DC, JN, EY, AP, and MG are employed by Adverum Biotechnologies, Inc.

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