Anc80 Delivery of the RPGRIP Gene Stabilizes Functional Vision Decline in a Genetic Mouse Model

Kathryn Post DeMott, PhD; Sharmila Vijay MA, MSc; Jennifer Klein, MSc; Ashley R Winslow PhD
Odylia Therapeutics

SCAN ME

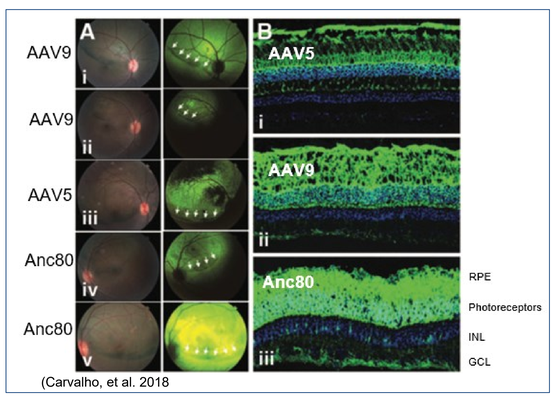
Abstract

Homozygous and compound heterozygous loss-of-function mutations in the *RPGRIP1* gene are associated with several rare retinal dystrophies, most commonly with the diagnosis of LCA type 6 (LCA6). LCA6 is characterized by early infantile onset, often with a lack of recordable cone electroretinogram (ERG) by 12 months of age. In most *RPGRIP1*-mediated vision loss, cone function is extinguished prior to rod function as seen in ERG recordings. This cone-then-rod function loss leads to reduced visual acuity followed by a reduction in the peripheral field. However, many patients appear to retain central retina laminar structure and architecture into adolescence and adulthood providing a potentially broad window for treatment with gene therapy with the possibility of regaining some photoreceptor function post treatment.

Odylia Therapeutics is developing a gene therapy (OT-004) to treat vision loss caused by *RPGRIP1* mutations. This gene therapy utilizes the Anc80 AAV capsid to deliver a functional copy of the *RPGRIP1* gene to photoreceptors. To-date, strong proof-of-concept data has been generated in a mouse model of LCA6, and a feasibility study conducted in non-human primates. The data supports efficacy and vector tolerability at the doses tested. Initial yield assessment data indicates promising manufacturing capabilities of this gene therapy.

Background

Anc-AAVs are synthetic adeno-associated viral vectors (AAVs), developed in the laboratory of Luk Vandenberghe (Carvalho et al., 2018; Zinn et al., 2015). Anc-AAV technology uses computational and evolutionary methods to predict novel conformations of the adeno-associated viral particle. Anc-AAVs share several features of naturally occurring AAVs and their variants. However, Anc-AAVs are fully man-made and do not occur in nature. Anc80, a putative ancestor of AAV1, 2, 3, 6, 7, 8, rh.10, and AAV9, was designed to retain key desirable aspects for clinical translation, while distinguishing itself antigenically from circulating AAVs.



(Carvalho, et al. 2018

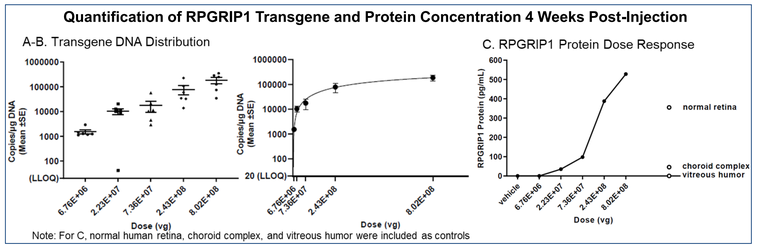
Anc80 was shown in mice and non-human primates to be a safe and potent therapeutic subretinal gene delivery vector in preclinical studies. Anc80 transduces cells of the INL and GCL and shows increased expression in RPE and photoreceptor layer compared to AAV2. Fundus exams shows faster onset of expression with Anc80 compared to AAV2 or AAV8 (Carvalho et al., 2018).

OT-004 uses Anc80 to deliver RPGRIP1 to photoreceptors. Retinitis pigmentosa GTPase regulator-interacting protein (RPGRIP) localizes to outer segments of human rod and cone photoreceptors as well as neuronal cells such as amacrine cells (Mavlyutovet al., 2002). RPGRIP localizes specifically to the photoreceptor connecting cilium, where it is essential for disk morphogenesis and regulation of actin cytoskeleton dynamics (Roepman et al., 2000).

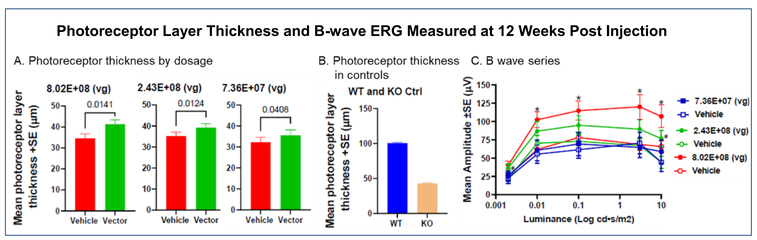
In the retina, RPGRIP1 was found to localize to 4 distinct regions (Koenekoop, 2005) :
1) photoreceptor outer segments,
2) amacrine cells of the inner retina,
3) cytoskeleton of photoreceptors, and
4) decorating microtubules

Efficacy Data in a Mouse Model

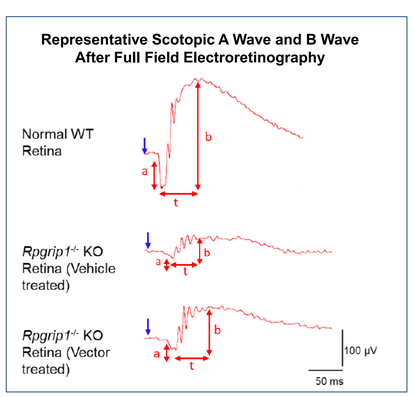
Wildtype (WT) mice were injected subretinally in both eyes between 4 to 6 weeks of age with vehicle or OT-004 at one of 5 different doses to determine ability of the vector to effectively deliver and express RPGRIP1. Five weeks post injection, the retina was dissected, and half the eyes were analyzed for human RPGRIP1 protein expression while the other half were analyzed by qPCR for transgene DNA. Injection of OT-004 in WT mice resulted in elevated dose-related levels of RPGRIP1 transgene and protein in the retina of mice 5 weeks post treatment.



In an RPGRIP1 knockout mouse, three groups of mice received subretinal injections of vehicle in one eye and OT-004, at three different concentrations in the contralateral eye. At 12 weeks post-injection, photoreceptor layer thickness was measured by Optical Coherence Tomography (OCT) and retinal function was measured by Electroretinography (ERG). Retinas receiving the OT-004 showed higher mean photoreceptor layer thickness than retinas receiving vehicle. Paired t-tests revealed the differences between vehicle-treated eyes and contralateral vector-treated eyes were significant at each vector dose level.

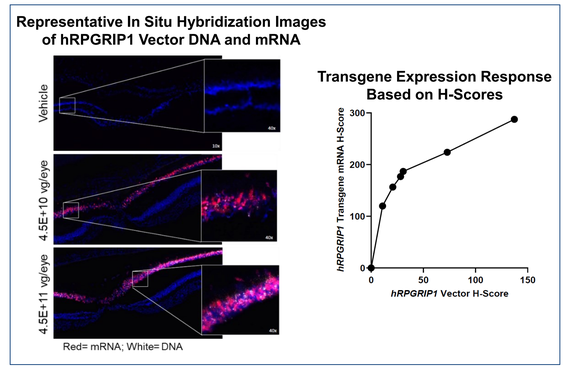


For the ERG B-wave series, mean amplitudes were increased for vector versus vehicle treated mice in a dose-dependent manner. Subretinal injections of OT-004 resulted in dose-dependent preservation of photoreceptor function by ERG and improved photoreceptor survival as measured by preserved retinal thickness by OCT at 12 weeks post treatment in RPGRIP1$^{-/-}$ KO mice.



Non-human Primate Tolerability Study

To evaluate the transgene expression and safety profile of OT-004, female cynomolgus monkeys were dosed with a single 150 µL single subretinal injection to both eyes. In-life measurements of intraocular pressure revealed a transient inflammatory response while OCT measurements found that the majority of effects observed after injection had resolved by 4 weeks post-injection. RPGRIP1 transgene expression was measured via in situ hybridization and quantified via HALO® imaging software (Indica Labs, Albuquerque, NM). The transgene expression response increased with increased levels of vector DNA.



Manufacturing

Manufacturing of OT-004 has commenced at Andelyn Biosciences using Andelyn's Suspension Platform. Initial yield assessment data, utilizing Andelyn's proprietary suspension cell line, provides promising support for the manufacturability of this gene therapy. OT-004 and a control vector of Anc80.eGFP in both lysis and non lysis conditions exceeded the typical minimum threshold of 2.5E10 vg/mL, previously not reached in adherent productions on a consistent basis.

Condition	Harvest Method	Average Titer
OT-004	Unlysed	1.11E11
	Lysed	9.85E10
Control (Anc80.eGFP)	Unlysed	1.31E11
	Lysed	1.32E11

Andelyn BIOSCIENCES Reliably Expert. Purposefully Driven.

Results provided by Sarah Bergman, BA; John Ketz, MSc; and Samir Acharya, PhD of Andelyn Biosciences.

Acknowledgements

We would like to acknowledge the work of Luk H. Vandenberghe, PhD, Eric Pierce, PhD, PTC Therapeutics, ACD Bio, Andelyn Biosciences, Labcorp, and Aldevron. We would also like to express our greatest appreciation for our donors and the entire RPGRIP1 community.

References:

- Carvalho L, et al. (2018). Synthetic Adeno-Associated Viral Vector Efficiently Targets Mouse and Nonhuman Primate Retina In Vivo. Human Gene Therapy. 29(7): 771-784.
- Koenekoop RK (2005) RPGRIP1 is mutated in Leber congenital amaurosis: A mini-review. Ophthalmic Genet 26:175–179.
- Mavlyutov TA, et al. (2002) Species-specific subcellular localization of RPGR and RPGRIP isoforms: implications for the phenotypic variability of congenital retinopathies among species. Hum Molec Genet. 2002; 11:1899-1907.
- Roepman R, et al. (2000)The retinitis pigmentosa GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. Hum Molec Genet. 2000; 9:2095-2105.
- Zinn E, et al. (2015). In Silico Reconstruction of the Viral Evolutionary Lineage Yields a Potent Gene Therapy Vector. Cell Reports 12: 1056–1068.