Evaluation of Ocular Tolerability of OPGx-RDH12 by Subretinal Delivery in Cynomolgus Primates

1621-B0205

Ashwath Jayagopal, PhD, MBA[1]; Mayur Choudhary, PhD[1]; Ben Yerxa, PhD[1]; George Magrath, MD, MBA, MS[1]

[1]Opus Genetics, Inc., Durham, NC, United States

Purpose

Leber congenital amaurosis (LCA) is a set of rare, genetically heterogeneous retinal dystrophies that lead to early-onset and severe retinal degeneration. LCA13 is caused by mutations in the retinol dehydrogenase 12 (RDH12) gene.[1,2] It encodes a key visual cycle enzyme, a short-chain retinol dehydrogenase/reductase, which is essential for photopigment regeneration in the visual cycle. The RDH12 enzyme provides reductase activity for reducing all-trans retinal (ATRAL) released after photoactivation. This step is essential for photopigment regeneration in the visual cycle. The regeneration process ultimately converts vitamin A to 11-cis retinal, the chromophore of rod and cone photoreceptors. As free ATRAL is cytotoxic, RDH12-mediated removal of ATRAL also protects the photoreceptor inner segments. It has been argued that the disease predilection for the central retina in these patients results from light-mediated degeneration and/or interference with the human retinoid cycle within this highly specialized retinal region.[3]

Approximately 1,125 people (1:288,333) in the U.S. have mutations in RDH12,[4] accounting for approximately 4% of all autosomal recessive inherited disease (IRD) cases. Patients with RDH12-IRD are typically symptomatic from early childhood with poor central vision, nystagmus, and reduced light sensitivity. Patients with early-onset severe RDH12-IRD are legally blind (i.e., poor visual acuity and limited visual fields) from early childhood and may become nearly totally blind between the second and third decade of life.[1,2]

OPGx-RDH12 is a recombinant, non-self-replicating adeno-associated virus 8 (AAV8) vector containing codon-optimized human RDH12 complementary DNA (cDNA) under the control of rhodopsin kinase 1 (RK1), a photoreceptor-specific promoter designed to restrict expression to the target cell type and drive expression levels comparable to those in photoreceptors.

The objective of this 30-day study in cynomolgus non-human primates (NHPs) was to determine the ocular tolerability of OPGx-RDH12 (AAV8-RK1-hoptRDH12), the test article designed to deliver the codon-optimized human RDH12.

Methods

Study Design:

Group No.	Treatment (OU)	Dose (vg/eye)	Dose Volume (vg/eye)	Dose concentration (µl/eye)	No. of animals
1	Vehicle	0	150	0	2
2	Low Dose	3 X 10^{10}		2.00 X 10^{11}	2
3	Mid Dose	1 X 10^{11}		6.67 X 10^{11}	2
4	High Dose	3 X 10^{11}		2.00 X 10	2

Species/Strain	NHP/Cynomolgus monkey
Age at initiation of dosing	2 years 3 months - 2 years 8 months
Body weight range at initiation of dosing	1.8 – 2.2 kg
Number of acclimation days	17 days

Husbandry: Housing set-up was as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2 and 3) and as described in the Guide for the Care and Use of Laboratory Animals (NRC, current edition). Animals were separated during designated procedures/activities or as required for monitoring and/or health purposes, as deemed appropriate by Study Director and/or Clinical Veterinarian.

Clinical Ophthalmic Examination (OE): OEs were conducted via indirect ophthalmoscopy and slit-lamp biomicroscopy at Predose, Days 7, 14, 21, and prior to termination. Uveitis scoring was also performed, as necessary, at the time of eye examinations.

Intraocular Pressure (IOP) Measurements: IOP was measured by rebound tonometry at approximately the same time of day to account for diurnal variation, at Predose, Days 7, 14, 21, and prior to termination.

Wide-field Color Fundus Imaging (CFI): CFI was performed in both eyes of all study animals at Predose, Day 1 and prior to termination.

Optical Coherence Tomography (OCT) Imaging: A single horizontal, high resolution line scan from the optic nerve head through the fovea, as well as a volume scan that included the dosing site, were performed at Predose, Day 1, and prior to termination.

Biodistribution (BD): Samples were collected from one eye (n=1 animal per group): Retina (bleb), Retina (non-bleb), RPE/Choroid (bleb), and RPE/Choroid (non-bleb).

Immunogenicity: Peripheral blood mononuclear cell (PBMC) samples were analyzed for the antigen-specific immune activation using a qualified method (Predose, Day 14, and terminal). An ELISpot assay was conducted to measure the cellular interferon gamma (IFN_{γ}) response and neutralizing anti-AAV8 and anti-RDH12 antibodies.

Histology: Eyes from all groups were trimmed, processed, embedded in paraffin, microtomed, and stained with hematoxylin and eosin (H&E). Ocular microscopic and macroscopic pathology evaluations were conducted.

Results

Table 1. Individual Ophthalmic Observations

Group	Sex	Animal	Day	Comment
1 – 0 vg/eye	Male	1001	8	Minimal pale tan RPE mottling with central bleb OS
	Male	1001	15	Minimal pale tan RPE mottling with central bleb OS
	Male	1001	22	Minimal pale tan RPE mottling with central bleb OS
	Male	1001	26	Minimal pale tan RPE mottling with central bleb OS
2 - 3 X 10^{10} vg/eye	Male	2001	8	Large fibrin clot on central cornea OD
	Male	2001	15	Bands of white/yellow inflammatory material within central vitreous, obscuring view of fundus OD.
	Male	2001	22	Bands of white inflammatory material within central vitreous, partially obscuring view of fundus OD.
	Male	2001	26	Bands of white inflammatory material within central vitreous, generalized hazy view of fundus, multifocal pigment dispersed over anterior lens capsule OD.
	Male	2002	7	Dark appearance of fovea OD.
	Male	2002	14	Dark appearance of fovea OD.
	Male	2002	25	Mild pale tan RPE temporal bleb OS.
3 - 1 X 10^{11} vg/eye	Male	3001	26	Minimal tan RPE mottling around retinotomy OS.
4 - 3 X 10^{11} vg/eye	Male	4001	26	Multifocal tan RPE inferior bleb OU.
	Male	4002	25	Mild pale tan RPE temporal peripheral bleb OU.

Figure 1. OCT Findings Limited to Procedural Effects on Retinal Structure

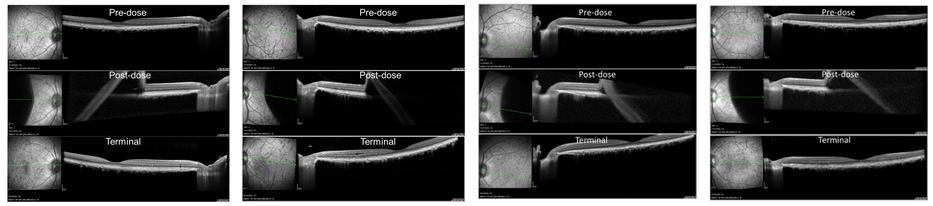
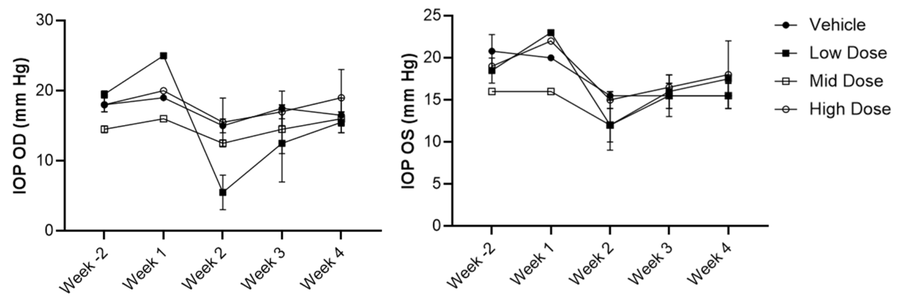


Figure 2. IOP Measurements Exhibited Normal Range for Sedate NHPs at all Timepoints



Immunogenicity Findings:

- No study sample tested in the ELISpot assay displayed a cellular IFNγ response above the assay limit of detection.
- Of the 8 animals tested across all timepoints, 4 were positive at all timepoints and did not have a significant decrease in normalized response suggesting that no additional neutralizing antibody activity was present post-dose; 3 of 8 animals tested across all timepoints had a negative NAb response at pretest, but were positive for NAbs at a minimum of 1 time point post-dose demonstrating a dose-related neutralizing antibody production.
- All groups tested negative for anti-RDH12 antibodies at all timepoints.
- 22/25 serum samples tested positive for anti-AAV8 antibodies (Pretest and Day 14 timepoints from all groups)

IFNγ , interferon gamma; IOP, intraocular pressure; NAb, neutralizing antibody assay OD, right eye; OS, left eye; OU, both eyes; RPE, retinal pigment epithelium.

Results

Table 2. Summary of OPGx-RDH12 Related Microscopic Findings

	Males			
Group	**1**	**2**	**3**	**4**
Dose (vg/eye)	**0**	**3x10^{10}**	**1x10^{11}**	**3x10^{11}**
No. Animals Examined	**2**	**2**	**2**	**2**
Eye, left (No. Examined)	2	2	2	2
Degeneration; retinal pigmented epithelium	(1)	(1)	(2)	(2)
Minimal	1	1	0	0
Mild	0	0	2	2
Hypertrophy; retinal pigmented epithelium	(1)	(1)	(2)	(2)
Minimal	1	1	0	0
Mild	0	0	2	2
Detachment; retina	(1)	(1)	(2)	(2)
Minimal	1	1	2	2
Eye, right (No. Examined)	1	1	1	1
Degeneration; retinal pigmented epithelium	(0)	(1)	(1)	(1)
Minimal	0	1	0	0
Mild	0	0	1	1
Hypertrophy; retinal pigmented epithelium	(0)	(1)	(1)	(1)
Minimal	0	1	0	0
Mild	0	0	1	1
Detachment; retina	(0)	(1)	(1)	(1)
Minimal	0	1	1	1

Conclusions

- OPGx-RDH12 was well tolerated, with no significant ophthalmoscopic findings and no systemic effects of the test article.
- A potential immune response to the AAV8 capsid was observed, typically peaking at Day 14, but with no apparent immunogenicity to the RDH12 protein.
- OCT evaluation revealed mild retinal structural changes at doses of 1 x 10^{11} or 3 x 10^{11} vg/eye that reflect delayed recovery of procedural effects and may be reversible with additional recovery time. All other OCT findings were related to the injection procedure.
- There were no clinically significant ophthalmoscopic or IOP findings at any dose level, aside from one eye in a low dose animal that developed moderate intraocular inflammation in the first week post-injection, which was considered a result of the injection procedure.

Based on available data, the no observed adverse effect level (NOAEL) for a single bilateral subretinal administration of OPGx-RDH12 in cynomolgus monkeys with a 30-day observation period was the high dose of 3x10^{11} vg/eye; Findings at mid and high dose levels were not considered adverse and likely attributable to incomplete recovery within the 30-day period following treatment.

References

1. Aleman TS, Uyhazi KE, Serrano LW, et al. *Invest Ophthalmol Vis Sci.* 2018;59:5225.
2. Aleman TS, Roman AJ, Uyhazi KE, et al. *Invest Ophthalmol Vis Sci.* 2024;65.
3. Sarkar H and Moosajee M. *Exp Eye Res.* 2019;188:107793
4. Stone EM, Andorf JL, Whitmore SS, et al. *Ophthalmol.* 2017;124:1314.

Disclosures: Ashwath Jayagopal, PhD, MBA (E); Mayur Choudhary, PhD (E); Ben Yerxa, PhD (E); George Magrath, MD, MBA, MS (E) of Opus Genetics, Inc.

Acknowledgements: This work was partially supported by the Foundation Fighting Blindness (FFB) and the Global RDH12 Alliance. The study was conducted at Charles River Laboratories (Mattawan, MI site). Immunogenicity sample analyses were conducted at Bioagilytix (Durham, NC).

Direct all inquiries to ajayagopal@opusgtx.com