Evaluation of MERTK Gene Therapy in RCS Rats Following a Single Bilateral Subretinal Injection

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Purpose

Retinitis pigmentosa (RP) is a set of clinically and genetically heterogeneous inherited retinal dystrophies (IRDs) characterized by progressive primary degeneration of rod photoreceptors leading to secondary degeneration of cones. RP typically manifests with difficulties in dark adaptation and night vision, followed by progressive peripheral visual field loss, with subsequent loss of central vision.[1]

Mutations in MER proto-oncogene tyrosine kinase (MERTK) result in approximately 3% of RP cases or 1:576,000 people. MERTK is a GAS6/ProteinS receptor involved in the phagocytosis of photoreceptor outer segments (POS) by retinal pigment epithelial (RPE) cells. *MERTK* mutations cause a rod-cone dystrophy with early macular atrophy, with RP being the most common retinal phenotype.[2] Treatment has the potential to greatly improve visual function and quality of life for affected individuals.

Preclinical studies have shown validation of early proof-of-concept of administration of *MERTK* gene therapy in Royal College of Surgeons (RCS) rats and demonstrated its safety.[3] Additionally, a Phase I dose-escalation study (NCT01482195) that tested this gene therapy approach in a small cohort of *MERTK*-related RP patients showed safety and potential efficacy.[4]

RCS rats were selected for this study based on their traditional use as an in vivo model for RPE dysfunction and resulting retinal degeneration. RCS rats are not transgenic and have a natural mutation of the *MERTK* gene, which leads to the loss of most photoreceptors by the age of 3 months.

The purpose of this study is to evaluate the effect of OPGx-MERTK gene therapy in a model of retinal degeneration in RCS rats following a single bilateral subretinal injection at P14.

Methods

Study Design:

Group (N)	Treatment (OU)	Regimen (OU)	Dose (vg/eye)	Volume (µL/eye)	Target Conc. (vg/mL)	Readouts: OE / OCT / MFI / ffERG	Termination and Tissues Collected
1 (13 M, 11 F)	Vehicle	Single Subretinal injection on Day 1 (PND 14±2)	NA	3 µL	NA	OE: Baseline, Day 1 post injection and prior to each termination	Day 20/21/22 N= 37 animals
2 (11 M, 11 F) Low dose	AAV	Single Subretinal injection on Day 1 (PND 14±2)	5E9		1.67E12	OCT & Fundus: Day 1 immediately post-injection and prior to each termination	Day 35/39/41/42/43 N=24 animals
3 (15 M, 11 F) Mid dose			1E10		3.33E12	(OCT with ONL thickness quantification at termination)	Day 69/71/72/75 N=35 animals
4(12 M, 12 F) High dose			5E10		1.67E13	ERG: prior to each termination	Whole globes were collected in fixative for histopathology analysis

Clinical Ophthalmic Examinations (OE): Slit lamp biomicroscopy and indirect ophthalmoscopy were performed on both eyes of all study animals at baseline (prior to test/control article administration), Day 1 post-injection, and prior to each termination

Optical Coherence Tomography (OCT): OCT imaging was performed, and outer nuclear layer (ONL) thickness was quantified in both eyes of all study animals on Day 1 (immediately post-injection), and prior to each termination

Multicolor Fundus Imaging (MFI): MFI was performed in both eyes of all study animals on Day 1 (immediately post-injection), and prior to each termination timepoint

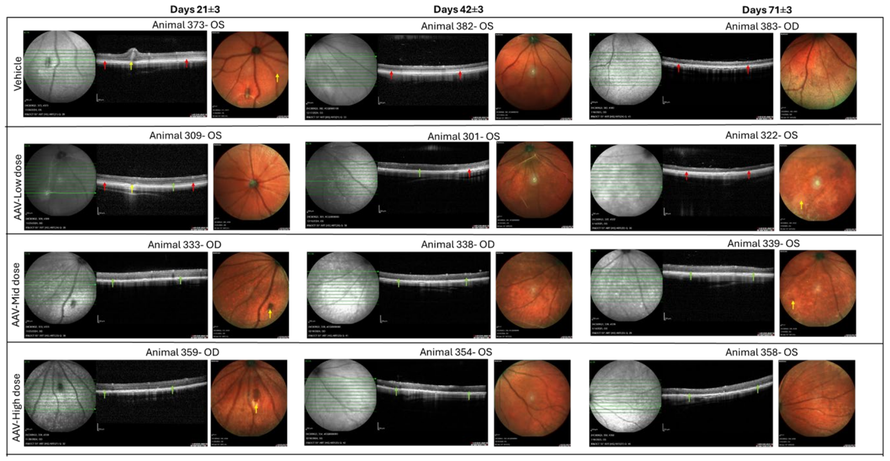
Full field Electroretinography (ffERG): ffERG was performed on both eyes of all study animals prior to each termination. Scotopic responses were obtained under dark conditions by following a 5-step intensity series from 0.0002 to 39.8 cd*s/m2 that were present to both eyes. With increasing intensity, the interstimulus interval between the sweeps increased. Photopic responses were obtained after 5 minutes of light adaptation by following a 3-step intensity series from 0.0084 to 39.8 cd*s/m2 presented to both eyes. With increasing intensity, the interstimulus interval between the sweeps increased. Responses of 1 to 10 flashes were averaged to generate ERG a and b waveforms at each flash intensity (with the exception of the 39.8 cd*s/m2 stimulus during scotopic conditions, which were presented as a single flash).

Histopathology Analysis: Eyes from all groups were trimmed, processed, embedded in paraffin, and microtomed. Duplicate slides were labeled with the following immunohistochemistry (IHC) assays: rhodopsin, R/G opsin, and MERTK. An additional duplicate slide for all eyes was generated and stained with hematoxylin and eosin (H&E).

Results

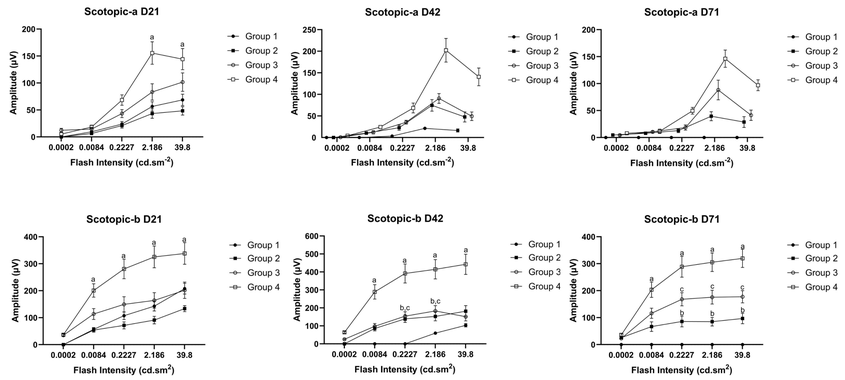
Figure 1. OPGx-MERTK Reduces Photoreceptor Debris in the Subretinal Space of RCS Rats

OCT and Fundus Observations



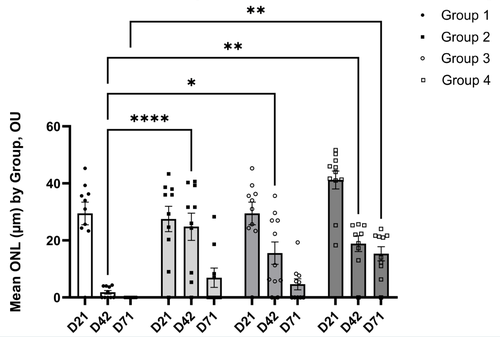
Yellow arrows represent the subretinal injection site. Red arrows show photoreceptor debris in the outer segments. Green arrows represent the preserved outer nuclear layer.

Figure 2. OPGx-MERTK Exhibits Dose-Dependent Preservation of Retinal Function in RCS Rats



a: Multiple t-tests Group 1 vs 4, b: Multiple t-tests Group 1 vs 3, c: Multiple t-tests Group 1 vs 2, p<0.01

Figure 3. OPGx-MERTK Exhibits Dose-Dependent Preservation of ONL Layer in RCS Rats



Results

Figure 4. OPGx-MERTK Treatment Leads to Dose-Dependent Expression of hMERTK in RPE Cells

MERTK-IHC

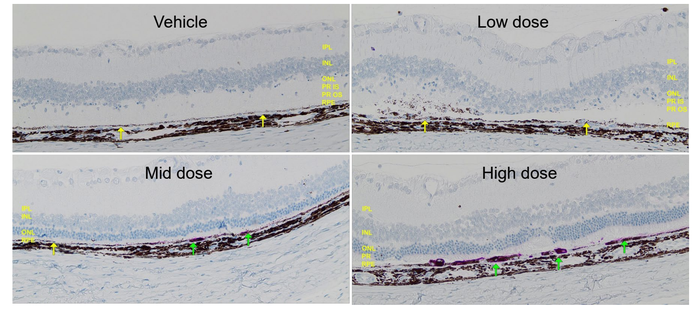
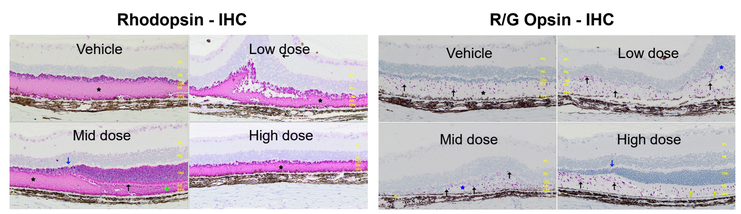


Figure 5. OPGx-MERTK-Treated RCS Rats Showed Normal Rhodopsin and R/G Opsin Localization



Conclusions

- There were no OPGx-MERTK dosing-related clinical observations or change in body weight in any treatment group.
- COEs were normal for all groups and findings of cornea opacity, lens cataract, and retinal scar at the injection site were related to either anesthesia or subretinal injection procedure.
- Vehicle-treated animals demonstrated typical RCS rat retinal phenotypes. Animals that received low dose, mid dose, and high dose of OPGx-MERTK had photoreceptor cells rescued (no debris deposition present) in ~2 retinal quadrants, more than 2 retinal quadrants, and more than 3 retinal quadrants, respectively.
- Retinal function analysis on ERG at Day 71±3 showed that b-wave amplitude was 3.6-fold higher in the high dose OPGx-MERTK group compared to the low dose group and 1.7-fold higher compared to the mid dose group at higher ERG intensity (39.8 cd•sec/m^2), suggesting that high dose OPGx-MERTK is more efficient in rescuing photoreceptor cells in RCS rats.

These findings suggest that treatment with OPGx-MERTK ameliorated photoreceptor degeneration and retinal deterioration characteristic of the RCS rat. Retinal function and survival of photoreceptor cells in dystrophic RCS rats was OPGx-MERTK dose-dependent and found to be more prominent with high dose OPGx-MERTK.

References

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