Nonclinical efficacy and toxicity study of GMP-grade vector OPGx-RHO (scAAV2/5-RHO820-shRNA820) delivered by subretinal injection in a canine model of RHO-adRP.

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Abstract#1435

Purpose

- Autosomal dominant retinitis pigmentosa (adRP) is an inherited, degenerative eye disease causing severe vision impairment and often blindness.
- Autosomal dominant RP accounts for 30% to 40% of all RP patients, and of these, up to 30% carry a mutation in the rhodopsin (*RHO*) gene. RHO-related adRP is estimated to be present in ~8,000 patients in the US and in ~12,500 patients in the EU.
- Proof of concept studies in a naturally occurring dog model of adRP, the closest disease model representative of human rhodopsin-mediated adRP, caused by a mutation in the *RHO* gene have recently shown that a knockdown and replacement gene therapy strategy confers protection to photoreceptors (both anatomic and functional) preventing retinal degeneration.

The objectives of this study were to evaluate the safety and efficacy of OPGx-RHO (scAAV2/5-*RHO*$_{820}$-shRNA$_{820}$) in rhodopsin (RHO) mutant dogs.

Methods

- Twelve RHOT4R/+ mutant dogs (4 males, 8 females) were divided into four treatment groups. At approximately 12 weeks of age, each dog received a 150 µL subretinal injection in the right eye. To maintain the integrity of the study, the animals were housed in dim red light from birth, and all retinal imaging was conducted using near-infrared light.
- At 11 weeks post-injection (PI), acute retinal degeneration was triggered in all 24 eyes via a one-minute light exposure (corneal irradiance of 1 mW/cm²). The study concluded at 27 weeks PI with the following evaluations:

  Toxicity Monitoring: This included weekly clinical observations and monthly check-ups covering clinical pathology, ophthalmic exams (intraocular pressure and ocular comfort), cSLO/OCT imaging, and terminal histopathology.

  Efficacy Assessment: It was measured by retinal function (via ffERG) and structural analysis—specifically outer nuclear layer (ONL) thickness and inner/outer segment (IS/OS) integrity—using OCT and histology.
- All animals were humanely sacrificed at the 27-week mark for final anatomic and histopathological analysis.

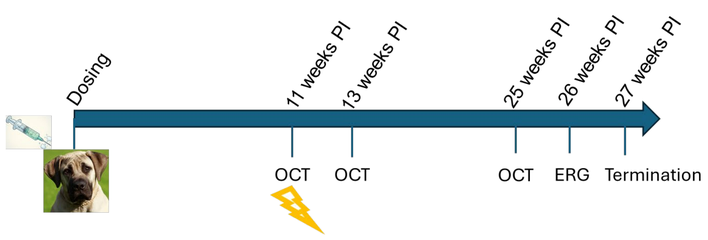


Table 1: Treatment groups

Vehicle	Low dose	Mid dose	High dose
BSS+0.014% PS20	1.5 X 10^{10} vg/eye	4.74 X 10^{10} vg/eye	1.5 X 10^{11} vg/eye
n=3, 1M, 2F	n=3, 1M, 2F	n=3, 1M, 2F	n=3, 1M, 2F

Results

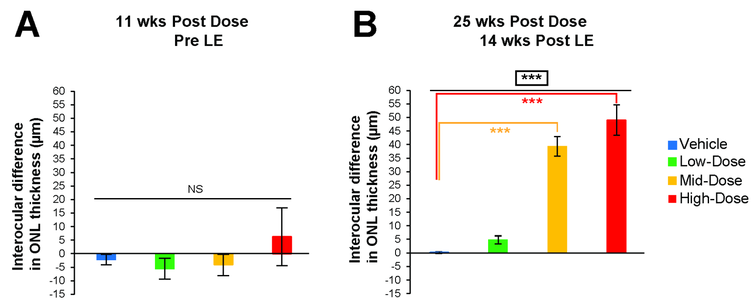


Figure 1. Comparison across treatment groups of the difference in ONL thickness (between treated and equivalent treated areas) from OCT-derived ONL maps.(**A**) At 11 weeks post dosage (pre-LE) and (**B**) at 25 weeks post dosage (14 wks post LE). Boxed asterisks represent p value of one-way ANOVA; colored asterisksrepresent pvalue of Bonferroni post hoc analysis***=p ≤0.001.LE= light exposure; NS= non-significant.

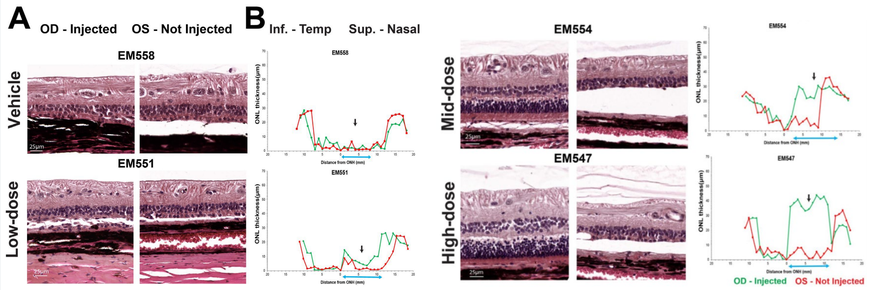


Figure 2. Representative retinal histology and quantification of ONL thickness at 27 weeks post dosage in individual injected and uninjected eyes from all 4 treatment groups. (A) Photomicrographs of H&E-stained sections showing the retinal morphology in the treated area of the injected (OD) eyes and the equivalent location of the contralateral uninjected (OS) eyes. **(B)** Spidergraphs of ONL thickness measured in both eyes(green traces= OD/injected eyes; red traces= OS/uninjected eyes) that extend from the optic nerve head (ONH) to the peripheral *ora serrata* along both the infero-temporal (Inf.–Temp.) and supero-nasal (Sup.–Nas.) quadrants. The section was oriented so as to include the treated area in OD and equivalent area in OS. The blue bar under the x axis of each spidergraph corresponds to the treated area (and equivalent area in OS). The black arrows point to the location where the H&E images shown in **(A)** were taken from.

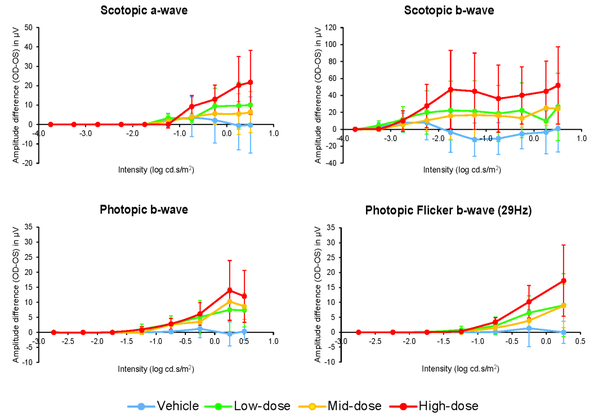
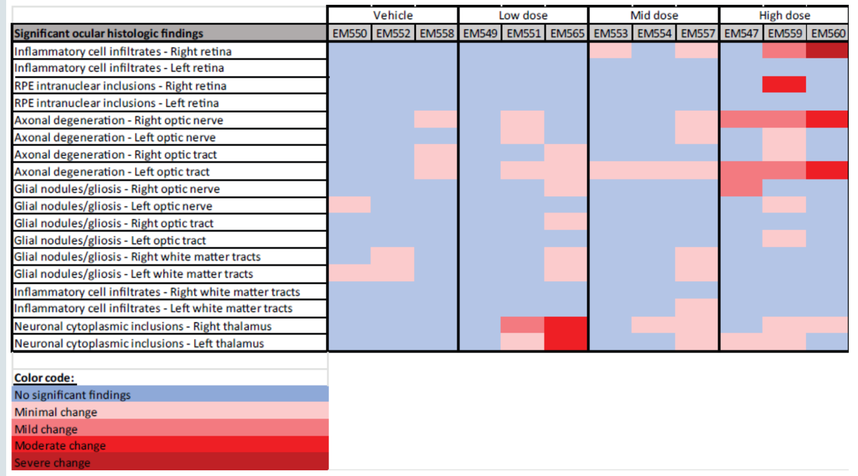


Figure 3. Comparison of ERG amplitudes across treatment groups at 26 weeks post dosage. Mean (±SD) differences in scotopic a- and b-wave, photopic b-wave and 29 Hz flicker amplitudes between the injected (OD) and uninjected (OS) eyes as a function of intensity of light stimulus. ANOVA did not show any statistically significant difference across groups.

Results



Significant ocular histologic findings	Vehicle			Low dose			Mid dose			High dose		
	EM550	EM552	EM558	EM549	EM551	EM565	EM553	EM554	EM557	EM547	EM559	EM560
Inflammatory cell infiltrates - Right retina												
Inflammatory cell infiltrates - Left retina												
RPE intranuclear inclusions - Right retina												
RPE intranuclear inclusions - Left retina												
Axonal degeneration - Right optic nerve												
Axonal degeneration - Left optic nerve												
Axonal degeneration - Right optic tract												
Axonal degeneration - Left optic tract												
Glial nodules/gliosis - Right optic nerve												
Glial nodules/gliosis - Left optic nerve												
Glial nodules/gliosis - Right optic tract												
Glial nodules/gliosis - Left optic tract												
Glial nodules/gliosis - Right white matter tracts												
Glial nodules/gliosis - Left white matter tracts												
Inflammatory cell infiltrates - Right white matter tracts												
Inflammatory cell infiltrates - Left white matter tracts												
Neuronal cytoplasmic inclusions - Right thalamus												
Neuronal cytoplasmic inclusions - Left thalamus												

Color code:
No significant findings
Minimal change
Mild change
Moderate change
Severe change

Figure 4. Heat map summary of ocular/visual pathway histopathological findings in all treatment groups.

Summary and Conclusions

- Preservation of ONL thickness in OPGx-treated retinas was observed.
- Clinical (non-ocular) observations and Clinical Pathology results did not reveal any findings that could be associated with the test-article.
- Ocular histopathology revealed signs of retinal inflammation (retinal perivascular lymphoplasmacytic cellular infiltration) in the mid and high dose of the test-article. In the mid-dose group, 2 out of 3 dogs had minimal perivascular inflammatory infiltrate. In the high-dose group, 2 out of 3 dogs had moderate to severe signs of retinal perivasculitis and 1 of those 2 animals also had retinitis.

These findings define the no-observed-adverse-effect-level (NOAEL) as the mid-dose of OPGx-RHO: 4.74 x 10^{10} vg/eye (3.6 × 10^{11} vg/mL).

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