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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Background

- Autosomal dominant Retinitis Pigmentosa (adRP) is an inherited, degenerative retinal disease, responsible of severe vision impairment that leads to blindness. adRP accounts for 30% to 40% of all RP patients,[1] and of these, up to 30% carry a mutation in the rhodopsin (RHO) gene.[2–5] RHO-related adRP is estimated to be present in ~8,000 patients in the US and in ~12,500 patients in the EU.[6,7]
- A naturally-occurring mutation within a canine model of RHO-adRP is caused by a C to G transversion at nucleotide 11 in the RHO gene.[8] This point mutation changes Thr-4 to Arg (T4R) and affects glycosylation and stability of RHO, yet the mutant T4R protein is expressed in rods and traffics normally to the outer segment.[8]
- RHO mutant dogs demonstrated a drastic loss of outer nuclear layer (ONL) thickness within 2 weeks following the acute light exposure (LEx), consisting of a 1-minute exposure to white light (approximate corneal irradiance of 1 mW/cm^2).[9] We also showed that maintaining these dogs under cycling (12 h ON/12 h OFF) dim red light prevents or delays the onset of degeneration,[10] thereby enabling modulation of the degenerative process.
- Proof of concept studies in this canine model have shown that a mutation-independent gene therapy that combines in a single AAV2/5 vector RHO knockdown (shRNA820) and replacement with a resistant human RHO cDNA (RHO820) confers protection to photoreceptors (both anatomic and functional), thereby preventing retinal degeneration.[11]

Purpose

To evaluate the safety and toxicity of a GMP batch of scAAV2/5-RHO820-shRNA820 vector (OPGx-RHO) delivered by subretinal injection in RHO$^{T4R/+}$ mutant dogs and establish the no-observed-adverse-effect-level (NOAEL) that will define the upper dose to be used in clinical trials.

Material & Methods

12 RHO$^{T4R/+}$ (4M/8F) mutant dogs were allocated to 4 treatment groups (**Table 1**). Subretinal delivery (150 µL) was performed in one eye (right) of each dog at >12 weeks of age. Dogs were kept under dim red-light illumination from birth until termination and retinal imaging was performed with near-infrared light only. All 24 eyes were light-exposed for 1 min with a corneal irradiance of 1 mW/cm^2, to trigger acute retinal degeneration, at 11 weeks post injection (PI). Toxicity was assessed by weekly clinical observations and monthly clinical pathology, ophthalmic examinations (ocular comfort and IOP), cSLO/OCT retinal imaging, gross anatomic pathology and histopathology. Efficacy assessment was based on retinal function (by ffERG), and outer nuclear layer (ONL) thickness and inner & outer segment (IS/OS) structure measured by OCT and histology. All animals were sacrificed 27 weeks PI.

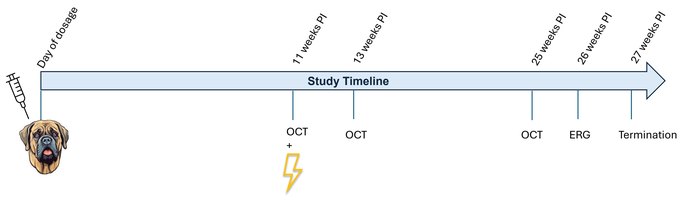

Table 1. Treatment groups

Vehicle	Low dose	Mid dose	High dose
BSS with 0.014% PS20	1.5 × 10^{10} vg/eye	4.74 x 10^{10} vg/eye	1.5x10^{11}vg/eye
	1.0 × 10^{11} vg/mL	3.16 x 10^{11} vg/mL	1.0x10^{12}vg/ml
3 dogs 1male, 2 females	3 dogs 1male, 2 females	3 dogs 1male, 2 females	3 dogs 1male, 2 females

Conclusions

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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1. Hartong, 2006
2. Sung et al, 1991
3. Inglehearn et al, 1992
4. Sohocki et al, 2021
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6. RetNet
7. Zhu et al, 2004
8. Meng et al, 2020
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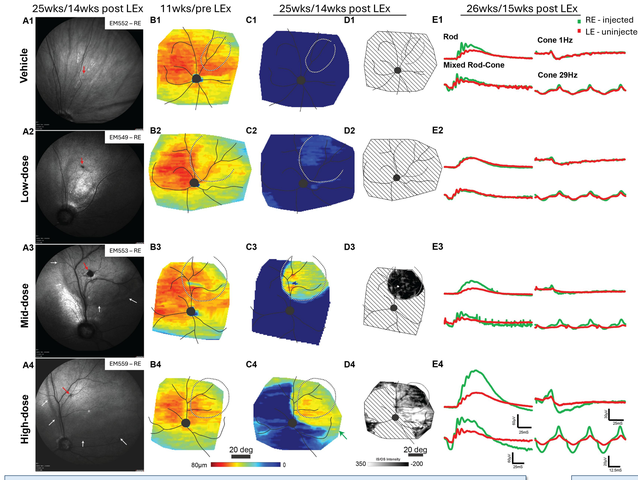
Results

Summary of structural and functional findings from one representative animal of each treatment group



A) Infrared cSLO images from the right eye (RE) at the end of study period (25 weeks post dose, 14 weeks post LEx) illustrating the structural rescue in the treated area (A2-4, red arrow indicates the retinotomy scar, white arrows pointing at the bleb border).

B) Pseudocolor ONL thickness maps of the RE based on OCT raster scans at 11 weeks post dose/pre LEx

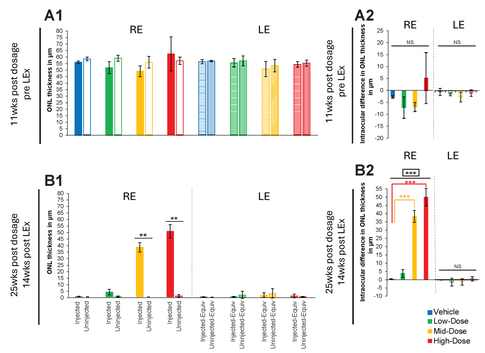
C) Pseudocolor ONL thickness maps of the RE based on OCT raster scans at the end of study (25 weeks post dose, 14 weeks post LEx). There is no structural rescue of ONL thickness outside the treated area in the test article-injected eyes (C2-4) and in the vehicle-injected eyes (C1). Note that in the high-dose injected dog (C4), there is ONL preservation in the periphery (green arrow) due to the uneven light exposure in the peripheral retina.

D) Normalized IS/OS intensity topography maps in the injected eye at the end of study (25 weeks post dose, 14 weeks post LEx). There is no detectable IS/OS signal outside the treated area in the test article-injected eyes (D2-4) and in the vehicle-injected eyes (D1).

E) ERG waveforms showing the rod (-1.74 log cd.s.m^{-2}), mixed rod–cone (0.5 log cd.s.m^{-2}) recorded dark-adapted, and cone responses to 1Hz stimuli (0.5 log cd.s.m^{-2}) or 29Hz cone flicker (0.25 log cd.s.m^{-2}) recorded under light adaptation in the injected (green trace) and uninjected (red trace) eye. Some retinal function in the vehicle, low-dose and untreated eye persists due to peripheral structural preservation. In mid (E3) and high dose (E4 groups, the retinal responses are higher in injected than in uninjected eyes.

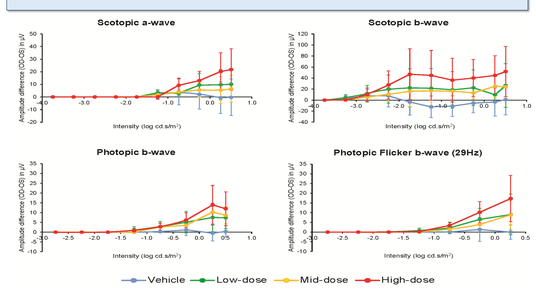
LEx: Light exposure

ONL quantification by OCT



ONL thickness analysis from OCT-derived ONL maps at 11 and 25 weeks post dosage.
A1) and **B1**) intraocular comparison within the mean (±SD) ONL thickness in treated and untreated areas of the injected right eyes (RE) and in equivalent treated and untreated areas in the uninjected contralateral left eyes (LE). Paired t-test; * = p ≤ 0.05, **= p ≤ 0.01
A2) and **B2**) Comparison across treatment groups of the mean (±SD) difference in ONL thickness between the treated and untreated areas of the injected eyes (RE) or equivalent areas in the contralateral uninjected eyes (LE).
Boxed asterisks represent p value of one-way ANOVA, colored asterisks represent p value of Bonferroni post hoc analysis * = p ≤ 0.05, **= p ≤ 0.01, and ***= p ≤ 0.001.
LEx = light exposure

Retinal function by ffERG



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 (RE) and uninjected (LE) eyes as a function of intensity of light stimulus.
ANOVA did not show any statistically significant difference across groups.

Ocular toxicity by histology

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

	Vehicle		Low dose			Mid dose			High dose	
Significant ocular histologic findings	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												
Inflammatory cell infiltrates - Right white matter tracts												
Inflammatory cell infiltrates - Left white matter tracts												

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

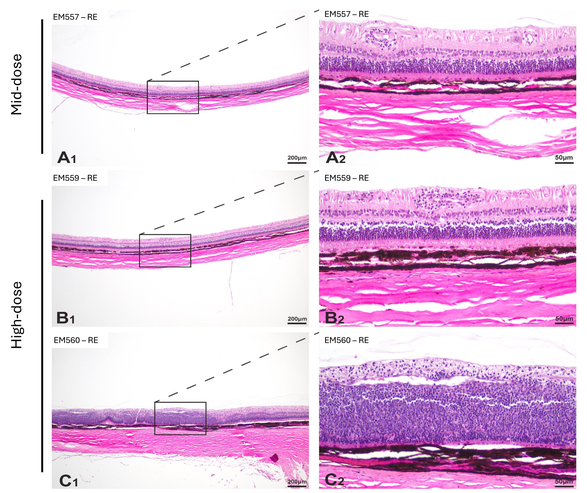
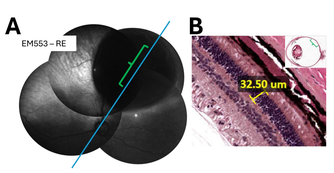
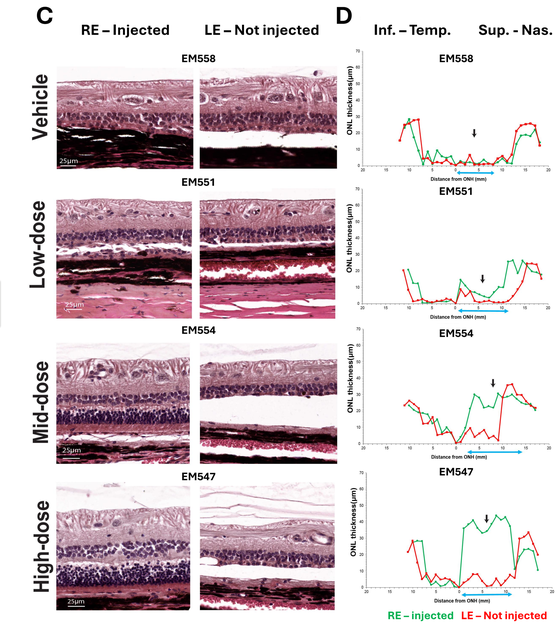


Illustration of different stages of retinal and chorioretinal lymphoplasmacytic cellular infiltration within the treated areas of eyes injected with test-article OPGx-RHO.
(A1-A2) Minimal retinal perivascular infiltrates in Animal ID EM557-RE (Mid-dose).
(B1-B2) Mild retinal perivascular infiltrates in Animal ID EM559-RE (High-dose).
(C1-C2) Severe retinal perivascular infiltrates in Animal ID EM560-RE (High-dose).

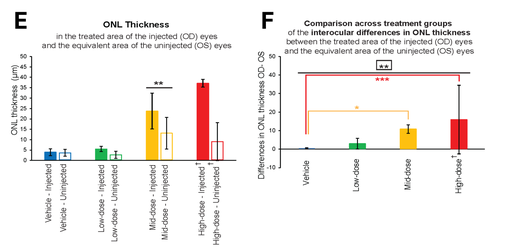
ONL quantification by histology



Globe dissection and illustration of ONL thickness measurement by histopathology
(A) Using the cSLO image of the bleb on the day of injection, sections were oriented to include the treated area in RE and the equivalent region in LE. (B) ONL thickness was measured every 1000µm along the entire retina. The green bracket represents the seven locations: one at the bleb center (black arrow (D); corresponding to the photomicrograph in [C]) and three adjacent points extending outward in each direction within the treated area and the corresponding region of the uninjected eye taken for quantification illustrated in (E) and (F).



Representative retinal histology and quantification of ONL thickness at study week 27 in individual injected and uninjected eyes from all 4 treatment groups.
(C) Photomicrographs of H&E-stained sections showing the retinal morphology in the treated area of the injected (RE) eyes and the equivalent location of the contralateral uninjected (LE) eyes.
(D) Spidergraphs of ONL thickness measured in both eyes (green traces = RE/injected eyes; red traces = LE/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 blue bar under the x-axis of each spider graph indicates the extent of the bleb in the injected area of RE (and the equivalent area in LE).



Quantitative analysis of the retention of ONL thickness in the treated area measured by histology at 27 weeks post dosage.
(E) Mean (±SD) ONL thickness of the treated area of the injected (RE) eyes and of the equivalent area of the uninjected (LE) eyes in the vehicle, low-, mid-, and high-dose treatment groups. Paired t-test; **= p ≤ 0.01
(F) Comparison across treatment groups of the mean (±SD) difference in ONL thickness between the treated area of the injected (RE) eyes and the equivalent area of the uninjected (LE) eyes.
Boxed asterisks represent p value of one-way ANOVA, colored asterisks represent p value of Bonferroni post hoc analysis; *= p≤0.05, **= p ≤ 0.01, and ***= p ≤ 0.001.