# National Institutes of Health (NIH), National Center for Advancing Translational Sciences (NCATS)

## Adeno-Associated Virus Nephrocystin 5 (AAV-NPHP5) for treatment of patients with NPHP5 Inherited Retinal Degeneration (IRD)

## Rare Pediatric Disease Designation Request

**June 18, 2024**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| aa | Amino acid |
| AAALAC | Association for Assessment and Accreditation of Laboratory Animal Care |
| AAV | Adeno-associated virus |
| AON | Antisense oligonucleotide |
| BB | Basal body |
| BrM | Bruch's membrane |
| BSS | Balanced Salt Solution |
| CC | Connecting Cilium |
| cDNA | Complementary DNA |
| COS | Cone outer segment |
| cSLO | confocal Scanning Laser Ophthalmoscopy |
| ERG | Electroretinogram |
| EZ | Ellipsoid zone |
| FDA | Food and Drug Administration |
| FDASIA | Food and Drug Administration Safey and Innovation Act |
| FST | Full-field stimulus test |
| GRK1 | G protein-coupled receptor kinase 1, Rhodopsin Kinase |
| IQCB1 | IQ motif containing B1 |
| IHC | Immunohistochemistry |
| IND | Investigational New Drug |
| IRBP | Interphotoreceptor retinoid-binding protein |
| IRD | Inherited Retinal Degeneration |
| IS/OS | Inner segment/outer segment junction |
| ISH | In situ hybridization |
| LCA | Leber Congenital Amaurosis |
| LRP | Longitudinal reflectivity profile |
| NIH | National Institutes of Health |
| nt | Nucleotide |
| NPHP5 | Nephrocystin 5 |
| OCT | Optical Coherence Tomography |
| OD | Right eye (oculus dexter) |
| OLM | Outer limiting membrane |
| ONL | Outer Nuclear Layer |
| OS | Left eye (oculus sinister) |
| PCC | Periciliary complex |
| PI | Post-injection |
| RPE | Retinal pigment epithelium |
| SRI | Subretinal injection |
| ss | Single stranded |
| sc | Self-complimentary |
| SRS | Subretinal space |
| vg | Vector genomes |
| WPRE | Woodchuck posttranslational regulatory element |
| WT | Wildtype |

# 1 RARE PEDIATRIC DISEASE DESIGNATION REQUEST STATEMENT

Pursuant to 21 CFR 316.20, the National Institutes of Health (NIH) requests designation of adeno-associated virus human Nephrocystin 5 (AAV-NPHP5) as a rare pediatric drug product for treatment of patients with inherited retinal degenerations (IRDs) due to mutations in the *NPHP5* (*IQCB1*) gene (*NPHP5*-IRD).

Inherited retinal degenerations are genetic conditions that cause vision loss that range in disease severity and age of onset (1). A particularly severe diagnosis within the IRD umbrella is Leber congenital amaurosis (LCA), which is a heterogenous group of monogenic diseases that cause childhood blindness (2). IRD due to biallelic mutations in the *NPHP5* gene (*NPHP5*-IRD) most commonly presents as LCA (3, 4). *NPHP5* encodes nephrocystin-5, a protein that is important in normal structure and function in photoreceptor cells of the retina (5). Patients are typically diagnosed in infancy or early childhood with profound, permanent vision loss (4-13).

Children with LCA and IRDs have significantly reduced health-related and vision related quality of life (14-18). Vision loss imposes challenges and limitations on daily activities, mobility and independence, and reduced vision from IRD has negative social and psychological effects on children; children often report feelings of anxiety, worry and frustration which follow through adulthood (14-20). They require special rehabilitation and schooling.

LCA as a group of diseases is rare. Estimates of prevalence of LCA range from 1:30,000 to 1:81,000 (21-23). *NPHP5*-IRD as a subset of LCA is very rare with a United States (US) prevalence estimate of ~1:346,000 and global prevalence ranging from ~1:600,000 to ~1:4,200,000 (21, 24-27). Only ~972 people are affected with *NPHP5*-IRD in the US (27). Very low prevalence qualifies *NPHP5*-IRD as a rare disease.

*NPHP5*-IRD is an autosomal recessive disease where visual acuity and light sensitivity of both rods and cones are severely reduced. There is early and rapid rod photoreceptor loss across the retina and the retention of a central island of cone photoreceptors at the fovea which persist over many years, at many disease stages which provide a wide therapeutic window (4, 8). Visual function is disproportionately affected relative to the remaining photoreceptor structure which indicates that visual improvement may be achievable upon therapeutic intervention. In this way, *NPHP5*-IRD is similar to LCA caused by mutations in *RPE65* (28), the only IRD for which there is an approved gene therapy, Luxturna (voretigene neparvovec-ryzl). Introduction of a functional copy of *NPHP5* is expected to cause improvements in vision, just as with Luxturna (29-38).

*NPHP5*-IRD meets the criteria to be considered a rare pediatric disease since it is a serious and debilitating disease that affects children from birth, and since it has extremely low disease prevalence. We are therefore requesting that AAV-NPHP5 be designated as a Rare Pediatric Disease Product to treat *NPHP5*-IRD.

# 2 ADMINISTRATIVE INFORMATION

## 2.1 Sponsor

National Institutes of Health (NIH)
National Center for Advancing Translational Sciences (NCATS)
9609 Medical Center Drive, Suite 1-E-162
Rockville, MD 20850

## 2.2 Primary and Alternate Contacts

Regulatory Correspondent

[redacted]

Sponsor Contact

[redacted]

Principal Investigator

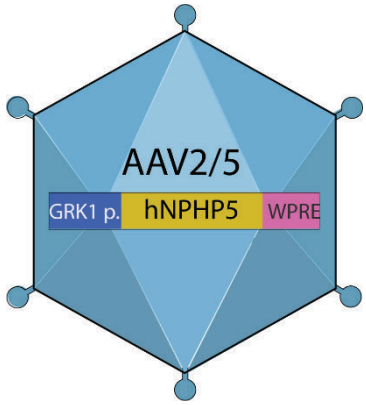
[redacted]

## 2.3 Drug Name

### *2.3.1 Chemical Name – Drug Substance*

Adeno-Associated Virus Pseudotype 2/5 human Nephrocystin-5 (AAV2/5-NPHP5).

### *2.3.2 Generic/Trade Name – Drug Product*




AAV-NPHP5 is a single stranded (ss) DNA sequence including a human rhodopsin kinase (GRK1) promoter, full-length human NPHP5 cDNA and a Woodchuck posttranslational regulatory element (WPRE) packaged into an adeno-associated virus pseudotype 2/5 capsid (ssAAV2/5-GRK1-hNPHP5-WPRE) (Fig.1). Additional details can be found in section 4.1.

**Figure 1: diagram of drug product (AAV-NPHP5)**

## 2.4 Manufacturer's Name and Address

### *2.4.1 Drug Substance Manufacturer*

Aldevron, LLC
4055 41st Ave South
Fargo, ND 58104
FEIN: 3015047170

### *2.4.2 Drug Product Manufacturer*

Andelyn Biosciences, Inc.
1180 Arthur E. Adams Dr.
Columbus, OH 43221
FEIN: 3026042970

# 3 DESCRIPTION OF RARE DISEASE OR CONDITION, PROPOSED INDICATION, AND NEED FOR THERAPY

## 3.1 Description of Rare Disease or Condition

Clinical Manifestations and Natural History

*NHPH5*-IRD is a rare, autosomal recessive inherited retinal degeneration that is predominantly characterized by severe and debilitating loss of vision from birth or early childhood. Most patients are typically diagnosed early in life, they are legally and profoundly blind, and they have a phenotype consistent with LCA. Parents often are the first to notice that their child is not seeing normally. Results from an electroretinogram (ERG) typically show severely reduced or extinguished rod and cone photoreceptor function in the retina. This result coupled with presence of nystagmus and inability to fixate, oculo-digital sign, keratoconus and/or abnormal

tracking and abnormal response to visual stimuli support the clinical diagnosis of LCA in young children (3, 4, 8). Genetic testing is then performed to confirm the clinical diagnosis and provide the causative gene. Best corrected visual acuities in patients with *NPHP5*-IRD are often worse than 20/200, visual fields are severely constricted, and patients exhibit decreased light sensitivity as measured by full-field stimulus testing (FST) (3, 4, 8). There is some variation in phenotype of *NPHP5*-IRD patients, and a minority of patients may have relatively preserved visual acuity, small central islands of vision or later onset of visual symptoms (3, 4, 8).

Detailed optical coherence tomography (OCT) imaging studies show that most *NPHP5*-IRD patients are born with rod photoreceptor cells but lose them to a severe time-course of retinal degeneration that is complete within the first decade of life such that no rod photoreceptors survive (4, 7, 8). Cone photoreceptors in *NPHP5*-IRD, on the other hand, manifest a very different course of disease. In *NPHP5*-IRD, there is a central island of structurally retained but dysfunctional cone photoreceptors. The outer nuclear layer (ONL), the layer containing photoreceptor nuclei, often persists for decades (8). These remaining photoreceptors would be the target of gene therapy, the goal of which would be to improve vision.

Visual impairment significantly reduces quality of life in children, and the effects are life-long (14, 16, 17, 19, 39). Children with IRDs score significantly lower on physical health and psychosocial health measures compared to their unaffected siblings, and score lower than children with other serious chronic conditions (14). Worse visual acuity, typical in patients with *NPHP5*-IRD, is correlated with poorer quality of life and worse family functioning (14, 15). Performing tasks of everyday life are affected by vision loss. Visual impairment has negative effects on the wider emotional well-being, mental health and social function of children, and these psychological effects follow through to adulthood with adults often experiencing anxiety, depression and worry stemming from their vision loss (16-20). Younger adults with vision loss have almost five times the risk of serious anxiety or depression compared to adults aged 65 and older (40). There is higher incidence of Autism Spectrum Disorder among children with congenital visual impairment, and children with early vision loss may also experience developmental, speech and social delays which can be helped by early intervention by specialists (2, 41). There is no cure or medical treatment for *NPHP5*-IRD, patients are counseled and referred to low vision clinics for rehabilitation services. Letters of support that describe the debilitating nature of *NPHP5*-IRD and its impact on those affected are included as Appendices 2 and 3.

Pathophysiology

Rod and cone photoreceptors have four major compartments: the outer and inner segments, the cell body, and the synaptic terminal (42) (Fig. 2A). As part of their specialization, the photoreceptors contain one of the longest sensory cilia known in mammalian cells, extending across the inner segment where most of the biosynthesis takes place, and outer segments where phototransduction takes place. Between the inner and outer segments of photoreceptors is the ciliary transition zone, also called the connecting cilium (CC), which is a narrow isthmus that has two interrelated functions: CC acts as a conduit for the enormous flux of proteins trafficked to and from the outer segment, and CC acts as a gate to compartmentalize proteins to the inner or outer segments (Fig 2. B,C). One of many macromolecular complexes at the CC is thought to consist of at least NPHP5 and CEP290 (aka NPHP6) proteins and possibly also including three other nephrocystins NPHP1, NPHP4, and NPHP8 (43-51). Mutations in *NPHP5* cause loss of function, and thus disrupt normal structure and function of the CC in photoreceptor cells (4). Retinal ciliopathies, diseases that affect the normal structure and/or function of the CC, can demonstrate dramatic heterogeneity in retinal phenotype. Shared retinal phenotype of *NPHP5*-, *CEP290*- and *NPHP1*-IRDs is very specific and differs dramatically from most other IRD phenotypes associated with ~300 other genes (8).

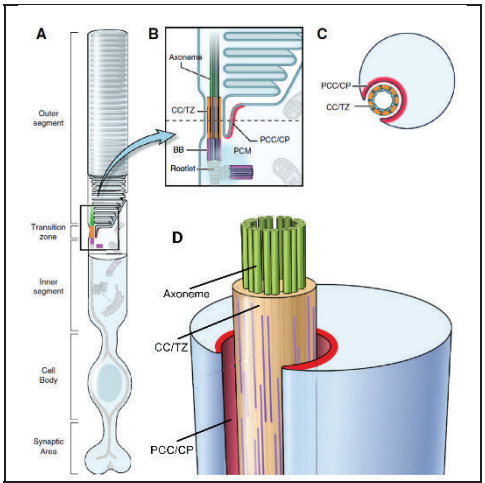



**Figure 2**: (**A**) Schematic of a photoreceptor, showing specialized domains of the cell. (**B**) Transition zone showing the structural and functional domains: axoneme (green), connecting cilium (CC; orange), basal body (BB; purple), periciliary complex (PCC; red). (**C**) Cross section through the CC. (**D**) 3D representation of the CC and adjacent domains. Modified from Rachel, Li and Swaroop, 2012.

## 3.2 Proposed Indication and Use of AAV-NPHP5

The proposed use of AAV-NPHP5 is to treat the retinal disease associated with mutations in *NPHP5*. The gene therapy vector expresses the full length human *NPHP5* (*hNPHP5*) gene under the control of a GRK1 promoter. The vector would be administered subretinally to the central retina, resulting in delivery of a functional copy of the protein to the photoreceptors. Translation of the wildtype (WT) protein is expected to correct the ciliary defect and restore the CC resulting in improved vision in patients. Since the degree of visual impairment is greater than what would be expected by the remaining retinal structure in these patients, restoration of the CC is anticipated to drive improved function of the remaining photoreceptors. Proof of concept studies conducted in a naturally-occurring canine model of *NPHP5*-IRD have demonstrated that *NPHP5* gene augmentation can prevent further loss of rod function and

restore cone function by enabling regrowth of cone outer segments that are absent shortly after birth.

## 3.3 Reasons Why Such Therapy Is Needed

*NPHP5*-IRD is a rare, autosomal recessive, severe, childhood-onset retinal disease for which currently no cures or treatments exist. The standard of care for this severe early-onset condition remains palliative only. The dissociation of photoreceptor structure and visual function make *NPHP5*-IRD a promising target for a gene therapy approach to intervention and suggests that therapy would result in vision improvement (52). The severe vision loss from *NPHP5*-IRD is debilitating, and children with vision loss due to IRD have lower quality of life compared to those who are normally-sighted which persists through adulthood (14, 17, 19, 20). In addition to difficulties performing daily life activities and visual navigation independently, vision loss has profound and lasting psychological and social impacts and is a source of life-long anxiety and worry (16-19). While IRDs leading to vision loss are rare, they are caused by molecularly simple single-gene defects, and are therefore good candidates for gene therapy. Despite this, there is only one approved gene therapy treatment for IRDs, Luxturna (voretigene neparvovec-ryzl), which is approved to treat LCA caused by biallelic mutations in *RPE65*. There remains a great need for similar therapies to treat other rare, inherited retinal conditions, such as *NPHP5*-IRD.

Treatment Potential

*NPHP5*-IRD is characterized by a central island of retained, albeit abnormally functioning cone photoreceptors. The amount of vision in these patients is disproportionately low relative to the retained central photoreceptor structure. Artificial intelligence that predicts visual function based on underlying retinal structure was used to show that there is treatment potential, the potential for improvement of visual function upon intervention, in patients with *NPHP5*-IRD (11). The dissociation of retinal structure and function in this disease, along with the prediction that there is treatment potential in this population, support the hypothesis that a successful molecular intervention, with the introduction of a wildtype copy of *NPHP5*, can result in quick and large improvements in visual function at the fovea in patients with *NPHP5*-IRD. The fact that the central cone island is retained for decades allows for an extended therapeutic window to treat the retinal disease.

Since *CEP290*-LCA and *NPHP5*-IRD share very specific phenotypic features and the gene products share cellular mechanism, efficacy results from *CEP290* treatment trials can be informative when predicting what can be expected upon treating *NPHP5*-IRD. Recent and ongoing trials use gene-based antisense oligonucleotide (AON) (Sepofarsen, NCT03140969, NCT03140969, NCT03913143) or gene editing (NCT03872479) approaches to treat the most common *CEP290* mutation (c.2991+1655A>G) (53, 54). Just as with *NPHP5*-IRD, correction of retinal ciliopathy in *CEP290* disease would be expected to improve dysfunctional cones.

Indeed, when cone light sensitivity was measured, nearly all treated eyes showed improvements in the Phase 1/2 clinical trial for Sepofarsen, the intravitreally injected AON (55-58). The Phase 2/3 trial for Sepofarsen failed to meet its primary outcome, although the reason why remains unclear. In the gene editing Phase 1/2 trial, results show improvement in visual function in BCVA, FST, visual navigation and vison-related quality of life (59, 60). As this gene therapy is delivered subretinally rather than intravitreally, the results from the gene editing trial could be more representative of what could be expected from subretinal administration of AAV-NPHP5.

Dissociation of structure and function, along with encouraging results from treatment of a similar disease offer hope for improved vision and quality of life for patients with *NPHP5*-IRD treated with AAV-NPHP5. Improved vision would change patients' lives. It would reduce the burden of vision loss on their own lives and the lives of their families and afford them opportunities in education and employment that might otherwise be unattainable. There is currently no approved therapy (or even ongoing clinical trials), and thus AAV-NPHP5 addresses a critical need for treatment that remains unmet for this severely affected, small population.

# 4 DESCRIPTION OF AAV-NPHP5 AND SCIENTIFIC RATIONALE FOR USE

## 4.1 Description of AAV-NPHP5

### *4.1.1 Drug Product Naming Convention*

Adeno-Associated Virus Pseudotype 2/5 human Nephrocystin-5 (AAV2/5-NPHP5).

### *4.1.2 Drug Product Physical, Chemical, and Pharmaceutical Properties*

Please refer to Appendix 1 for the complete nucleotide sequence.

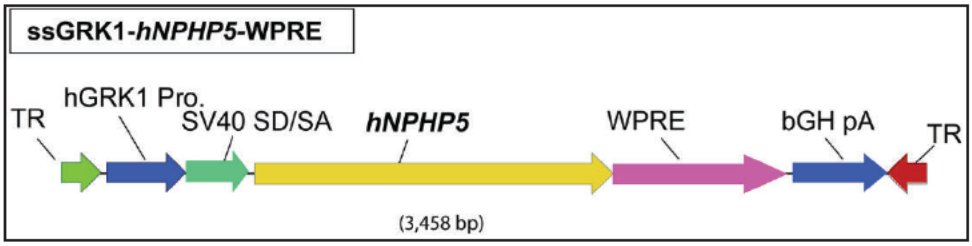



**Figure 3: Diagram of vector**

**Table:** Basic Elements and length composing the single stranded vector construct.

| Basic elements | Length (nt) | Information |
|---|---|---|
| 5' ITR | 143 | AAV2 Inverted terminal repeat |
| hGRK1 pro | 292 | Human rhodopsin kinase promoter |
| SV40 SD/SA | 164 | Simian Virus 40 splice donor/Splice acceptor element |
| hNPHP5 | 1,797 | Transgene CDS |
| WPREsf | 587 | Woodchuck Hepatitis virus posttranscriptional Regulatory Element |
| bGH/pA | 236 | Bovine Growth Hormone Poly A signal |
| 3' ITR | 143 | AAV2 Inverted terminal repeat |

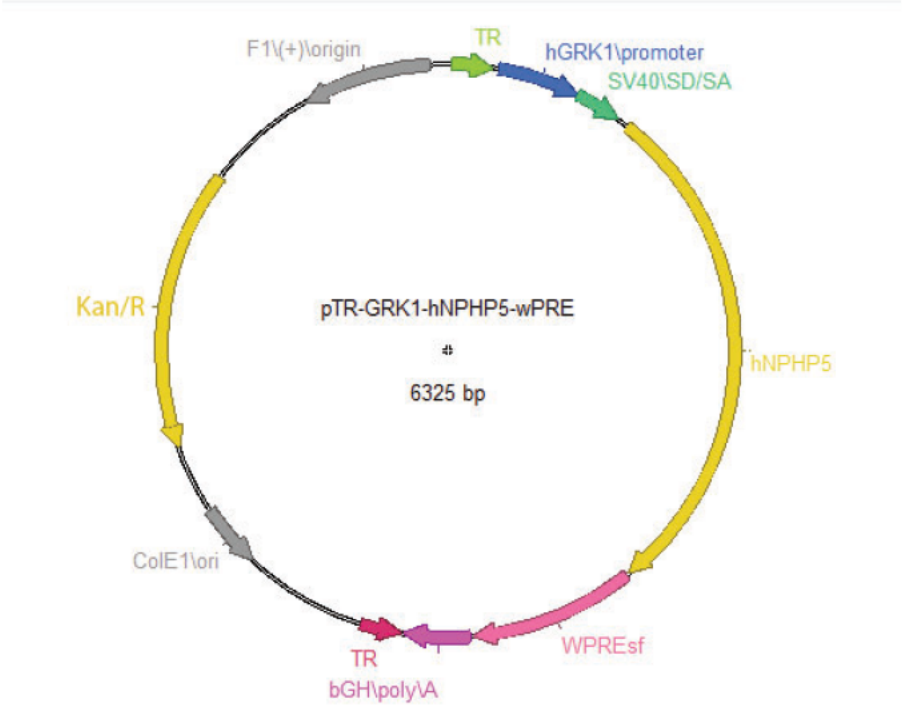



**Figure 4: Diagram of the vector plasmid map**

### *4.1.3 Drug Product Description, Mechanism of Action and Route of Administration*

The drug product is a single stranded AAV transgene expression cassette containing full length human NPHP5 cDNA and a Woodchuck Hepatitis virus posttranscriptional Regulatory Element driven by the human GRK1 promoter and packaged into an AAV2/5 capsid. The drug product will be formulated in Balanced Salt Solution (BSS) containing 0.001% Poloxamer 188, pH 7.0. It will be delivered via a subretinal injection, specifically targeting the retained central cone photoreceptors.

Briefly, following standard 3 port pars plana core and posterior vitrectomy, AAV-NPHP5 will be delivered to the macular area via a transvitreal subretinal injection. The tip of the subretinal cannula will be guided to the region of the vascular arcades and a superior retinotomy will be created, avoiding the blood vessels. The retinotomy should not be placed closer than 3 mm to the fovea. Viscous fluid injection pressure should be set to a maximum PSI of 16 mmHg, and the initial bleb formation should be attempted with as low of a PSI as possible (under 10 mm Hg). At the discretion of the surgeon a small (< 30 µL) pre-bleb with BSS can be made prior to vector delivery. 300 µL of the AAV-NPHP5 vector solution will be injected slowly and a subretinal bleb formed in the clinical macula (including the fovea). Intraoperative OCT images will be obtained when possible and evaluated during the subretinal bleb formation to monitor the status of the fovea. The bleb should not touch or approximate the optic nerve. Pre-, peri-, and post-operative corticosteroids will be administered to control for possible inflammation resulting from the vector.

An AAV vector is used to deliver the gene to the photoreceptors. AAV vectors have been well established as a safe mechanism of gene delivery. Introduction of the wildtype transgene will correct the ciliary defect, which results in the regrowth of the photoreceptor sensory cilium (outer segment) restoring its function and improving the overall function of the photoreceptors.

## 4.2 Scientific Rationale for the Use of AAV-NPHP5 in the Rare Disease or Condition

*NPHP5*-IRD is caused by biallelic mutations in the *NPHP5* gene. NPHP5 interacts with many other proteins that function in forming and supporting the CC in photoreceptor cells, therefore mutations result in severe loss of visual function and rod photoreceptors. Central cone photoreceptors are retained over decades, albeit functioning at levels lower than expected based on the retained retinal structure. Since there is a dissociation of retinal structure and function in this disease, correction of the ciliary defect by introduction of wildtype *NPHP5* is expected to result in improvement of visual function. A naturally occurring dog model of the disease was used to show the efficacy of AAV-NPHP5 in restoring visual function in affected animals at various disease stages and doses (7, 61). This model recapitulates the human form of the disease,

and so it is thought that administration of this therapy to humans would yield similar efficacy results.

### *4.2.1 Clinical Efficacy of AAV-NPHP5*

There is no clinical data collected on AAV-NPHP5 to date, as the first-in-human clinical trial has yet to begin.

### *4.2.2 Nonclinical Efficacy of AAV-NPHP5*

Background Information

*NPHP5* gene augmentation delivered by AAV vector prevents ongoing photoreceptor loss and restores cone structure and function in the NPHP5 mutant dog, a naturally-occurring canine model of early-onset *NPHP5*-IRD.

Initial (published) studies evaluated the efficacy of two different AAV serotypes with or without capsid modifications (AAV2/5; AAV2/8(Y733F)), containing one of two photoreceptor-specific promoters (interphotoreceptor retinoid-binding protein (IRBP), or GRK1) to drive expression of either the canine or human *NPHP5* cDNA (61). The four gene therapy constructs that included either the canine or human full length *NPHP5* cDNA under control of the GRK1 promoter were packaged as self-complementary (sc) DNA in AAV capsids. The decision to initially test scAAV-*NPHP5* constructs was based on the rapid kinetics of rod loss in this model and the need for rapid transgene expression to halt disease progression when treatment was delivered at the onset of degeneration (~ 5 weeks of age). Results showed that irrespective of the AAV capsid (AAV2/5, AAV2/8 (Y733F)) and transgene (human or canine *NPHP5*), early-stage gene therapy intervention prevented rod loss, allowed regrowth of cone outer segments, corrected mislocalization of rod, M/L and S opsin, and restored rod- and cone-mediated ERG function, as well as visual behavior in an obstacle avoidance course under scotopic and photopic conditions. The gene therapy was administered subretinally to NPHP5 mutant dogs at early-stage disease (~ 5-6 weeks of age). Signs of rescue were detectable within 8 weeks post-injection and remained stable throughout the 6-month posttreatment period. Efficacy (preservation of ONL thickness, reformation of cone outer segment structure, restoration of rod and cone-mediated ERG and visual behavior) was found to be independent of the AAV serotype used and transgene expressed, however GRK1 appeared to be a more potent promoter than IRBP. Stock solutions of the viral vectors were formulated in BSS with 0.014% Tween (polysorbate) -20, pH 7.0 and further diluted with BSS to titers that ranged from $1.5 \times 10^{11}$ to $4.7 \times 10^{12}$ vg/mL (doses: $1.1 \times 10^{10}$ to $3.3 \times 10^{11}$ vg/eye). Efficacy was detected even with the lower titers/doses (61).

Follow-up studies assessed long-term efficacy of NPHP5 dogs treated with different scAAV-GRK1-NPHP5 vectors at early-, mid-, late- and very late-stage disease and showed stable functional rescue for 3-5.4 years (62). Extensive clinical (confocal scanning laser ophthalmoscopy (cSLO)/OCT) and histological/immunohistochemistry (IHC) assessment of

treated eyes did not reveal any signs of toxicity nor ocular inflammation even with viral vector titers as high as 4.76 x $10^{12}$ vg/mL (Dose 7.1 x $10^{11}$ vg/eye).

Due to concerns with sequence heterogeneity in scAAVs preparations (scAAVs can contain single stranded DNA, and are more prone to package plasmid backbone sequences compared with ssAAVs) (63, 64), the human *NPHP5* transgene was recently packaged under control of the GRK1 promoter together with a Woodchuck posttranslational regulatory element as a single stranded DNA into an AAV2/5 capsid. With the goal of translating this gene therapy into human clinical trials, a series of experiments both in WT and homozygous mutant NPHP5 dogs was more recently conducted to confirm the efficacy of this investigational product (ssAAV2/5-GRK1-hNPHP5-WPRE).The following sections provide a brief description of the animal model, the methodology used, and a summary of the salient findings of these proof-of-concept nonclinical studies conducted at the University of Pennsylvania, School of Veterinary Medicine between 3-March-2021 and now.

Animal model

The NPHP5 mutant dog is naturally-occurring model of *NPHP5*-IRD that is homozygous for a single nucleotide insertion in exon 10 of *NPHP5*. The mutation results in a 12 amino acid (aa) frameshift, and premature stop that truncates the C-terminal 268 aa of the 598 aa native protein (65). The mutation triggers an early onset and rapidly progressive retinal degeneration with a preservation of non-functional central cones that recapitulates the spatiotemporal pattern of the *NPHP5*-IRD described in people (Fig. 5 and (7)). In this canine ciliopathy, cones fail to form outer segments from birth, and rods, while initially structurally intact rapidly undergo degeneration. Based on the thickness of the ONL at different ages, the following stages of disease have been established in the canine model: early-stage (ONL loss ~ 0%; 5-6 weeks of age); Early/mid stage (ONL loss: 25-30%; ~8 weeks of age); Mid stage (ONL loss: ~30-40%; 12-14 weeks of age); Late stage (ONL loss > 50%; > 24 weeks of age). Dogs with late-stage disease retain an ONL along the cone-rich visual streak and specifically within the temporally located area centralis. A comparable spatial pattern of disease can be observed in 13-15 year-old NPHP5 patients that have

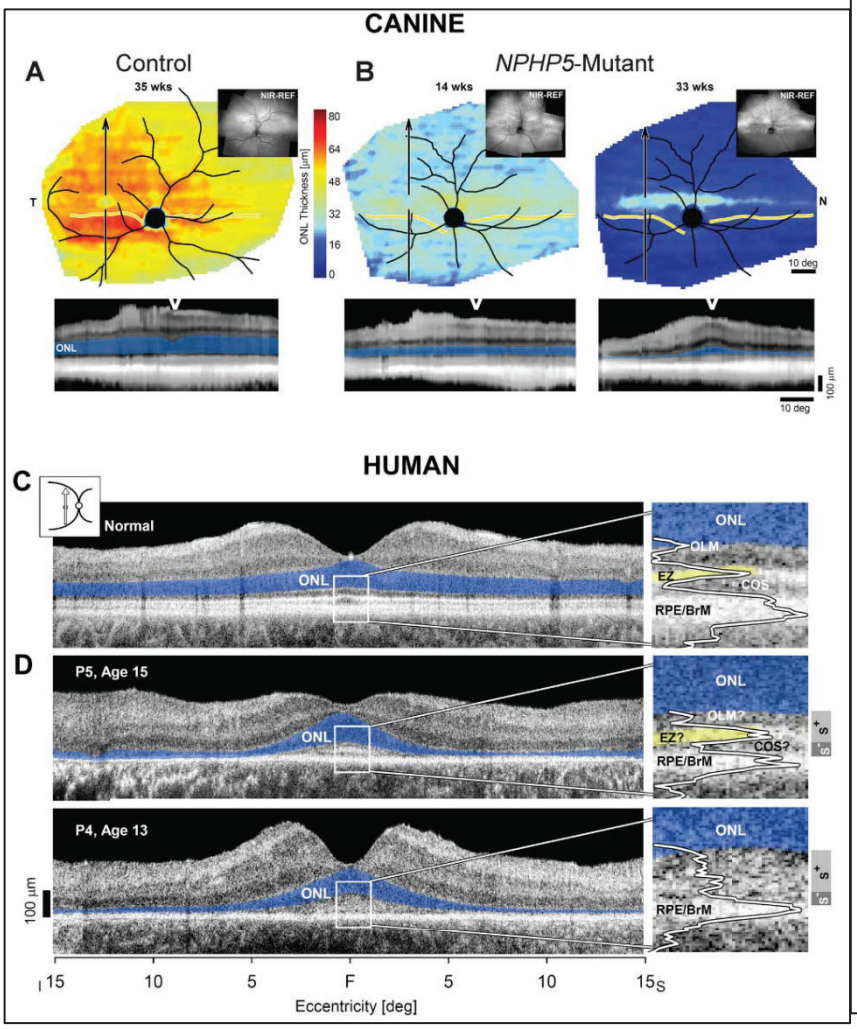



**Figure 5: Canine and human NPHP5 retinal structure**. (**A-B**) Pseudocolor maps of ONL thickness topography (upper) in a 35-week-old control dog (A) and a NPHP5-mutant dog (B) at 14 and 33 weeks of age. Insets, near-infrared reflectance images. Arrows on the maps localize the reconstituted OCT scans (lower) along a superior–inferior meridian crossing the central visual streak at the gaps of the lines. ONL on reconstituted scans is highlighted in blue and location of the visual streak is shown with a white wedge.
(**C-D**) Cross-sectional OCT scans along the vertical meridian through the fovea, extending 15 deg into superior (S) and inferior (I) retina in (A) a normal subject (age 30 years) and (B) NPHP5-IRD patients P5 and P4 (ages 15 and 13 years, respectively). Magnified views of the central retina (right; white rectangles) with overlapping longitudinal reflectivity profiles (LRP). Photoreceptor nuclear layer (ONL) is highlighted in blue; EZ (ellipsoid zone) is highlighted in yellow. OLM, outer limiting membrane; COS, cone outer segments. RPE/BrM, retinal pigment epithelium/Bruch's membrane. In the patients, there are wide hyperscattering bands ($S^+$) and more narrow hyposcattering bands ($S^-$) distal to the ONL (far right along image). Modified from Downs et al., 2016.

ONL retention in the fovea.

A unique research colony of NPHP5 mutant dogs has been established at the Retinal Disease Studies facility (Division of Experimental Retinal Therapies, School of Veterinary Medicine, University of Pennsylvania), an animal facility that is AAALAC accredited.

Efficacy assessment methods

Regular ophthalmic examinations including biomicroscopy, tonometry, indirect ophthalmoscopy, and fundus photography were performed at baseline and throughout the in-life period to monitor post-operative changes including funduscopic signs of retinal preservation.

Non-invasive retinal imaging using cSLO and OCT with a Spectralis Heidelberg HRA/OCT2 unit was performed at baseline and at various timepoints post injection (PI), to examine the effect of gene therapy intervention on retention of ONL thickness.

Full-field ERG (Espion $E^3$, Diagnosys LLC electrophysiological system) was performed at baseline and at various timepoints post injection to assess the effect of gene therapy on rod -, cone-, and mixed rod/cone-mediated function.

Visually-guided behavior was tested under scotopic, mesopic and photopic environments (8 different conditions ranging from 0.003 to 646 Lux) in a custom-made obstacle avoidance course. Uniocular testing was conducted by masking the contralateral eye with an opaque occluder. Transit time and number of collisions were measured from video recordings by a masked examiner.

In situ hybridization (ISH) was performed on paraformaldehyde fixed, cryoprotected and OCT embedded retinal tissues using NPHP5 probes (RNAscope™) to detect expression of the human *NPHP5* transgene.

Efficacy of AAV-NPHP5 in dogs

A summary of the unpublished results of the proof-of-concept studies conducted at the School of Veterinary Medicine, University of Pennsylvania to this date with the ssAAV2/5-GRK1-hNPHP5-WPRE viral vector is shown in the Table below and in Appendices 4-7.

| Study | Dog ID | NPHP5 Genotype | Eye | Vector titer (vg/mL) | Volume injected in SRS (in uL) | Stage of disease at time of SRI | Longest post SRI follow-up (in weeks) | Transgene expression (RNA-ISH) | Clinical signs of ocular toxicity/ inflammation | ONL rescue (OCT imaging) | ERG rescue | Visual behavior rescue |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study #1**: Transgene expression in WT | RC718 | WT/WT | OD | $4.76 \times 10^{12}$ | 100 | N/A | 8 | Yes | No | N/A | N/A | N/A |
| | | | OS | $4.76 \times 10^{11}$ | 100 | N/A | 8 | Yes | No | N/A | N/A | N/A |
| **Study #2**: Efficacy at Early/Mid stage disease | AS2-447 | Mut/Mut | OD | $4.76 \times 10^{11}$ | 110 | Early/mid | 116 | N/A* | No | Yes | Yes | Yes |
| | WM66 | Mut/Mut | OS | $4.76 \times 10^{11}$ | 50 | Early/mid | 101 | N/A* | No | Yes | Yes | Yes |
| **Study #3**: Efficacy at Late Stage Disease | AS2-448 | Mut/Mut | OD | $4.76 \times 10^{11}$ | 150 | Late | 102 | N/A* | No | Yes | Yes | Yes |
| **Study #4**: Identifying Therapeutic Range | WM62 | Mut/Mut | OS | $4.76 \times 10^{10}$ | 150 | Mid | 96 | N/A* | No | Yes | Yes | Yes |
| | WM63 | Mut/Mut | OS | $1.0 \times 10^{11}$ | 150 | Mid | 96 | N/A* | No | Yes | Yes | Yes |
| | WM67 | Mut/Mut | OS | $4.76 \times 10^{11}$ | 70 | Mid | 97 | N/A* | No | Yes | Yes | Yes |

N/A: non applicable
*: Study ongoing

Study #1: Efficacy of hNPHP5 transgene expression in photoreceptor cells was first established in a WT dog that was subretinal-injected with two doses OD: $4.76 \times 10^{12}$ vg/mL, and OS: $4.76 \times 10^{11}$ vg /mL; subretinal injection (SRI) vol: 100 µL) of ssAAV2/5-GRK1-hNPHP5-WPRE (see Appendix 4).

Study #2: Long term efficacy of AAV-NPHP5 in mutant NPHP5 dogs was then evaluated in 2 NPHP5 mutant dogs that received an SRI of ssAAV2/5-GRK1-hNPHP5-WPRE in one eye ($4.76 \times 10^{11}$ vg/mL; SRI vol: 50-110 µL) at early/mid stage disease. Structural preservation (ONL detected by OCT imaging in the treated area), functional improvement (retention of some rod-mediated function and recovery of cone-mediated function detected by ERG), as well as improved visual performance under photopic, mesopic and photopic illumination in an obstacle avoidance course were observed for > 2 years (see Appendix 5).

Study #3: Long-term efficacy of AAV-NPHP5 was subsequently evaluated in a mutant NPHP5 dog that received an SRI of ssAAV2/5-GRK1-hNPHP5-WPRE in one eye ($4.76 \times 10^{11}$ vg/mL; SRI vol: 150 µL) at late-stage disease. Mild structural preservation (ONL detected by OCT imaging in the treated area), functional improvement (recovery of cone-mediated function detected by ERG), as well as improved visual performance under photopic, mesopic and photopic illumination in an obstacle avoidance course were observed for up to 2 years (see Appendix 6).

Study #4: was conducted to determine the therapeutic range of ssAAV2/5-GRK1-hNPHP5-WPRE by assessing efficacy and safety in 3 NPHP5 mutant dogs subretinally-injected

uniocularly at mid-stage disease (~ 12 weeks of age) with one of three doses (Low: 4.76 x$10^{10}$; Mid: 1.0 x $10^{11}$; High: 4.76 x $10^{11}$ vg/mL; SRI volume: 70 -150 µL). The overall goal being to identify the lowest dose that still provides some efficacy.
Funduscopic examination showed retention of retinal vasculature (mid and high doses) and reduction of tapetal hyperreflectivity (a clinical sign of retinal degeneration in dogs) only in the high-dosed animal.

OCT imaging revealed of mild to pronounced retention of ONL thickness in the treated retinal area of all 3 dogs that was dose-dependent.

Retinal function assessed by full-field ERG showed a transient recovery of cone function as early as 12 weeks PI in the low-dosed animal. In the mid-dosed animal, cone-mediated function was first seen at 8 weeks PI, and although decreased, was still detectable at 84 weeks PI. Earlier restoration of cone function (4 weeks PI) that was also associated with restoration of rod function (8 weeks PI) was seen in the high-dosed animal and sustained at 84 weeks PI.

Visually-guided behavior assessed using a multiluminance obstacle avoidance course showed improved performance under photopic (cone-stimulating) conditions in the low-dosed animal. Improved visual performance was also seen in the mid-dosed dog when testing the treated eye under, mesopic (rod and cone stimulating) and photopic (cone stimulating) conditions. In the high-dosed animal improvement was seen when testing under scotopic (rod-stimulating). mesopic (rod and cone stimulating) and photopic (cone stimulating) conditions. In all three dogs the positive effect on restoration of visual behavior was seen up to > 90 weeks PI. There were no clinical signs of ocular toxicity/inflammation noted during that period (see Appendix 7).

Conclusions
The results of these proof-of-concept studies confirm prior work (61, 62) that showed that AAV-mediated NPHP5 gene augmentation rescues the disease phenotype in a clinically-relevant large animal model of *NPHP5*-IRD. Efficacy of ssAAV2/5-GRK1-hNPHP5-WPRE was demonstrated with a viral titer as low as 4.76 x $10^{10}$ vg/mL. This will provide useful guidance for the selection of doses to be tested in the context of a future IND-enabling efficacy and safety study.
Functional readouts (restoration of cone ERG function could be detected as early as 4 weeks PI. Intervention in NPHP5 mutant dogs at a late stage-disease that resembles that observed in teenage NPHP5 patients was efficacious. Efficacy was sustained for > 96 weeks PI. Although no histological assessment has yet been performed in any of the mutant NPHP5 dogs enrolled in these studies, clinical assessment has not revealed any signs of treatment-related ocular inflammation or toxicity.

## 5 ORPHAN DRUG STATUS

AAV-NPHP5 is not currently designated as an Orphan Drug to treat *NPHP5*-IRD or any other indication in the United States. We are seeking Orphan Drug Designation for this gene therapy.

## 6 PATIENT SUBSET CONSIDERATIONS AND MEDICAL PLAUSIBILITY OF THE CHOSEN SUBSET

*NPHP5*-IRD is an extremely rare disease; we are not requesting use of this investigational product for a subset of affected individuals. This section does not apply to this therapy.

## 7 REGULATORY STATUS AND MARKETING HISTORY

AAV2/5-NPHP5 is not currently marketed or approved for use in any country, including the United States. In accordance with section 526(a)(1) of the Federal Food, Drug, and Cosmetic Act [21 USC 360bb(a)(1)]; 21 CFR 316.20(b)(7); and 21 CFR 316.23(a), NCATS has not previously submitted a marketing application to FDA for the same active moiety in AAV2/5-NPHP5 for the same rare disease or condition prior to submission of the AAV2/5-NPHP5 rare pediatric disease designation request.

This therapy is currently in the late stages of pre-clinical development. There have been no interactions with the FDA to date regarding this investigational product, but we preparing to schedule a pre-IND meeting with the FDA to discuss the planned IND enabling toxicity and pharmacology studies and review the completed proof of concept preclinical studies. We are targeting IND application submission for fall of 2025.

## 8 DOCUMENTATION OF PATIENT POPULATION SIZE

*NPHP5*-IRD is a very rare condition with prevalence estimates ranging from 1:4,185,000 to 1:346,000. It is estimated that 972 individuals are affected by *NPHP5*-IRD in the United States. A PubMed literature search was performed, identifying prevalence estimates of *NPHP5*-IRD. Since *NPHP5*-IRD is very rare, sources examining populations outside of the US were also included.

The table below summarizes estimates of prevalence, largely based on cohort data collected by IRD-clinics, with the exception being a study that analyzed large genomic databases to estimate the number of individuals affected with *NPHP5*-IRD globally (24-27, 66, 67). The total population of the US used in the calculations is 336,442,310 (https://www.census.gov/popclock/).

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| Stone et al., 2017 (27) | Estimated prevalence for US: 1:346,000 (972 people) | 5 probands in 1,000 consecutive probands studied (760 of which were molecularly clarified) in a clinic in the US were found to have IRD due to mutations in *NPHP5*. The data were collected from consecutive families, and represented individuals from 40 of the 50 states, so the data, although collected at one clinic, could be considered representative of the US population. Extrapolating from a known prevalence of *ABCA4* in the US (1:10,000), the prevalence of *NPHP5*-IRD in the US is estimated to be 1:346,000. Based on the US population at the time, the authors estimated the total number of people to have *NPHP5*-IRD in the US to be 938. The total number of people born annually in the US with *NPHP5*-IRD was estimated to be 12. Using an updated US population of 336,442,310, the total number of affected individuals is estimated to be 972. |
| Hanany et al., 2020 (24) | Estimated global prevalence: 1:1,390,273<br><br>Estimated prevalence for US: 1:448,069 (751 people) | Based on large genetic databases, the incidence of those affected by *NPHP5*-IRD was calculated for 6 major global subpopulations, and based on their populations, estimated number of affected individuals was calculated. From these calculations, the authors estimated that 5,467 individuals globally were affected by *NPHP5*-IRD at the time of publication. This equates to a prevalence of 1:1,390,273 (or a frequency of $7.19 \times 10^{-7}$). Note that subpopulation frequencies for those affected by *NPHP5*-IRD ranged from $2.01 \times 10^{-9}$ (those in Finland) to $2.79 \times 10^{-6}$ (for those in Europe).<br><br>Based off of these data, and using the US Census US and World population clock (https://www.census.gov/popclock/) and the 2020 census population data (https://www.census.gov/quickfacts/) we can very roughly estimate the prevalence of *NPHP5*-IRD in the US, using the total US population of 336,442,310. Approximately 75.5% of those living in the US are White. If using the proportion from Hanany et al., 2020 for European descent ($2.79 \times 10^{-6}$), then approximately 710 individuals in the US (of European descent) are affected. 13.9% of the US population is black. Using the proportion of affected *NPHP5*-IRD for African ($5.01 \times 10^{-7}$), approximately 23 people in the US of African descent are affected. The average proportion of Asian populations (South Asian and East Asian) is $8.28 \times 10^{-7}$. If 6.3% of the population in the US is of Asian descent, then approximately 18 affected people of |

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| | | Asian descent in the US. Totaled, there are roughly 751 people affected in the US, which would equate to a prevalence of 1:448,069. This figure is comparable to the Stone et al., 2017 estimate. |
| Pontikos et al., 2020 (66) | Estimated prevalence for UK cohort: 1:605,454<br><br>Estimated prevalence in the US: 556 people | Pontikos et al., 2020 analyzed three cohorts of their IRD clinic in the United Kingdom (UK). The main study retrospectively analyzed 4236 individuals with molecularly clarified diagnoses from 3195 families (the 'main cohort'). They also studied a pediatric subpopulation ('pediatric cohort') consisting of 452 individuals from 411 families and a third cohort of patients who were seen within the last 2-3 years by the clinic (the 'current cohort'). *NPHP5* was responsible for causing disease in 11 families in the 'main cohort' and 5 families in the 'current cohort,' which accounts for ~ 0.34% of families in each respective cohort. In the pediatric cohort, *NPHP5* was disease-causing in 5 families, which is 1.23% of the families analyzed in the cohort. *NPHP5*-IRD is diagnosed early in life, and thus accounts for a greater proportion of pediatric patients compared to the populations that include adults.<br><br>In order to roughly estimate prevalence using this data, we will assume that, as is in Stone et al., that the incidence of *ABCA4* disease-causing mutations in the UK is also 1:10,000. The Stone et al., 2017 paper analyzed the proband per family, and so we will use the number of families in this calculation. Using the 'main cohort' data, the number of families with *ABCA4*-IRD was 666. If 666 families represent a prevalence of 1:10,000, then it can be understood that 11 families with *NPHP5*-IRD represents a prevalence of 1:605,454. Applying this prevalence to the US population, approximately 556 individuals are affected in US. |
| Karali et al., 2022 (26) | Global estimate of prevalence:<br><br>1:4,185,000,<br><br>or<br><br>1:2,675,000<br><br>Estimated prevalence in | Karali et al., 2022 retrospectively analyzed an Italian cohort consisting of 2790 individuals diagnosed with an IRD, which were followed by a single eye clinic. 2036 patients were molecularly clarified. Of those who were molecularly clarified, *NPHP5*-IRD accounted for 2 individuals. 2 of the total 2790 patients is 0.072%. This paper gives the general prevalence of all IRDs to be 1:3,000. If we use this global figure for IRD prevalence, then 0.072% of the 1 in 3000 who are affected would be attributed to mutations in *NPHP5*. This would equate to a prevalence of 1:4,347,826, which, if applied to the US |

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| | the US: 80 or 126 people | population, would estimate a prevalence of 80 individuals.<br><br>If instead, we used the assumption that *ABCA4* mutations have a frequency of 1:10,000, and *ABCA4* caused disease in 535 individuals in the cohort of 2790 individuals as given in the paper, then it can be calculated that *NPHP5*-IRD has a prevalence of 1:2,675,000. When applied to the US population 126 people would be estimated to have *NPHP5*-IRD in the US. Although much lower prevalence is estimated based on this study, the figures are similar to the global estimates from Hanay et al., 2020. |
| Zobor et al., 2023 (25) | Estimated prevalence in German cohort: 1:1,680,000 – 1:630,000<br><br>Estimated prevalence in the US: 200 – 534 people | This group analyzed a German cohort that was diagnosed with Leber congenital amaurosis. Of the 105 individuals analyzed, *NPHP5* was found to cause disease in 5. Based on the estimates given of the prevalence of LCA ranging from 1:30,000 and 1:80,000 given in this manuscript, it can be calculated that the range of *NPHP5*-IRD prevalence is approximately 1:1,680,000 and 1:630,000. In the US, this would mean that 200-534 individuals would be affected. This estimate of prevalence aligns with estimates from other sources. |
| Lin et al., 2024 (67) | Estimated prevalence in UK cohort: 1:715,000<br><br>Estimated prevalence in the US: 470 people | 4415 patients from 3953 families who were molecularly diagnosed with an IRD and seen at a single clinic in the UK between 2003 and 2020 were analyzed in this retrospective analysis. Twelve families had mutations in *NPHP5*. Using the methodology in Stone et al., 2017, assuming that *ABCA4* causes disease in 1:10,000 in a general population, and it was found that 858 families had *ABCA4*-IRD in this cohort, then the prevalence of *NPHP5*-IRD is estimated to be 1:715,000, and 470 individuals are expected to be affected in the US. This estimate is also similar to others, all of which estimate prevalence to be less than 1,000 affected in the US. |

There are limitations to these estimates. Since this is such a rare disease with very little information regarding prevalence, many estimates are derived from clinics in Europe, outside the United States. It is assumed that the prevalence calculated outside the US could be directly applied to the US population; however, populations outside the US may not represent the demographics of the US population and thus may not accurately reflect the prevalence of *NPHP5*-IRD in the US. This may explain some of the variability in estimates that were calculated from different sources. Additionally, many estimates are based on the prevalence of *ABCA4*-IRD, which may be variable in different countries. Regardless of these limitations, the

rarity of this disease is apparent. The most conservative estimate of the number of individuals in the US that are affected is from a source that is derived from the US population, and so it can be assumed to be the most reflective of the actual prevalence. Using this estimate, 972 people in the US are affected by *NPHP5*-IRD.

# 9 RARE PEDIATRIC DISEASE DESIGNATION JUSTIFICATION

Based on the evidence presented above, pursuant to Section 908 of FDASIA, and consistent with the Rare Pediatric Disease Priority Review Vouchers Guidance for Industry, the National Institutes of Health requests that AAV-NPHP5 be designated as a Rare Pediatric Drug Product for the treatment of *NPHP5*-IRD.

*NPHP5*-IRD is a serious and debilitating disease that primarily affects pediatric populations; children are affected from birth or early childhood and exhibit profound and permanent visual impairment that significantly reduces quality of life for those affected (3, 4, 8, 14, 17-19, 40). There is currently no approved treatment or cure. *NPHP5*-IRD is an extremely rare form of IRD, with only approximately 972 individuals affected in the United States (27) and 5,400 affected individuals globally (24).

Retained retinal structure in these patients is disproportionately better relative to the severe level of visual impairment, which suggests that this disease, if treated, could result in visual improvement (52). AAV-NPHP5 is a gene therapy investigational product that would deliver a functional copy of the gene to photoreceptors, correcting for the ciliary defect, and thus restoring function in the remaining photoreceptors of these patients.

Gene therapy for *NPHP5*-IRD is expected to improve photoreceptor signaling and this effect can be measured clinically as an improvement in light sensitivity. We expect this type of efficacy outcome both in pediatric as well as adult patients. More challenging will be improving higher level features of vision, such as visual acuity. In *CEP290*-IRD, patients with childhood-onset bilateral blindness who were treated decades later as adults have not recovered high level vision (57). Attainment of visual perception is a gradual process driven by visual experience over many months to years of life during early postnatal neurodevelopment. In non-human animals, lack of visual experience in the early postnatal period is known to result in major deficits in neuroanatomy and neurochemistry which are only partially reversible when vision is regained beyond a critical period (68-71). Translation of these experimental findings to human vision loss and later recovery has been studied extensively in amblyopia with early monocular visual impairment and in congenital bilateral dense cataracts with early binocular blindness (72-74). The conclusions are that treatment of severe visual deprivation must be corrected early during a window of cortical plasticity for good outcomes. Therefore, we believe AAV-NPHP5 treatment of *NPHP5*-IRD will demonstrate best outcomes in vision recovery in pediatric subjects both in terms of intervention occurring before the loss of majority of the photoreceptors and allowing normal development of the visual brain.

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# 11 APPENDIX 1: NUCLEOTIDE SEQUENCE OF HUMAN NPHP5 CDNA INCLUDED IN SSAAV2/5-GRK1-HNPHP5-WPRE VECTOR:

ATGAAGCCAACAGGTACAGACCCAAGGATCTTATCTATAGCTGCTGAAGTTGCAAAAAGCC
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GGAAGCTCAGAGTTGAAGAAAATCAAACAAGATATATATTGTTATGATCTCATTCAATATTG
CCTCTTGGTCCTCAGTCAAGATTATTCTCGAATCCAGGGTGGTTGGACTACAATTTCCCAGCT
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ACCTTAG

**National Institutes of Health (NIH)**

**AAV-NPHP5 for treatment of patients with *NPHP5*-IRD**

**Rare Pediatric Disease Designation Request**

## APPENDICES

Appendix 2: Letter of support from clinician specializing in IRDs

Appendix 3: Letter of support from patient advocacy group

Appendix 4: NPHP5 transgene expression in WT dogs treated with AAV-NPHP5

Appendix 5: Efficacy of AAV-NPHP5 in mutant NPHP5 dogs treated at early/mid-stage disease

Appendix 6: Long term efficacy of AAV-NPHP5 in a mutant NPHP5 dog treated at late-stage disease

Appendix 7: Therapeutic range and long-term stability of AAV-NPHP5 in mutant NPHP5 dogs.

Perelman School of Medicine
University of Pennsylvania

Department of Ophthalmology
Scheie Eye Institute

**Tomas S. Aleman, MD**
*Irene Heinz Given and John La Porte Given*
*Professor of Ophthalmology*

Office of Orphan Products Development
Food and Drug Administration
WO32-5295
10903 New Hampshire Ave.
Silver Spring, MD 20993

May 24, 2024

RE: AAV-NPHP5 RPDD application

Dear Members of the FDA,

Leber congenital amaurosis (LCA) is an umbrella name given to a group of genetic forms of blindness with a symptomatic onset of near total blindness before the year of age. Pathogenic mutations in *NPHP5 (ICBQ1)*, is a molecularly specific form of inherited retinal degeneration (IRD) that most commonly presents as this condition. It is particularly aggressive in terms of the profound vision loss that the patients and their families have to cope with. The blindness resulting from *NPHP5*-IRD remains without a causative treatment in the clinic.

The presence of highly dysfunctional, nearly silent, surviving photoreceptors, despite the severe vision loss, together with proof-of-concept in a large animal model of the disease have raised hope that a treatment may restore the cilia of the surviving photoreceptors and improve vision in our patients. This is the main rationale for performing retinal gene therapy in patients with NPHP5-IRD. The patient population that will ultimately be the ideal beneficiaries will be children. Without treatment, these patients, who are legally blind as infants, have a very different future. Correction of their genetic blindness may allow for mainstream education and employment, for example. Window of retino-cortical development for normal vision closes relatively early in life. Correction of the primary genetic defect and restoration of the function of photoreceptors will ensure that proper developmental processes, including cognition, take place normally.

With easy access to scientific information, parents are often well aware of the work towards treatments and know of existing proof-of-concept. In the clinic, one of the hardest tasks for a clinician is to explain to families that despite clear signs of treatment efficacy in animal models of these blinding diseases, we do not have a clinical trial to move towards a treatment of their loved ones. This is especially painful when they realize one of the reasons is the low prevalence of a given disease, such as *NPHP5*-IRD. The hope is that Rare Pediatric Disease Designation will expedite treatments for our patients, particularly children. Although an infrequent disease, treatment success in this IRD will likely impact other genetic neurodegenerations that remain similarly without a cure.

Sincerely,

[redacted]

HOPE in FOCUS
P.O. Box 705 | Ledyard, CT 06339
860-266-6062 | info@hopeinfocus.org
www.hopeinfocus.org

May 30, 2024

Office of Orphan Products Development
Food and Drug Administration
WO32-5295
10903 New Hampshire Ave.
Silver Spring, MD 20993

**RE:** Rare Pediatric Disease Designation for NPHP5-IRD

Dear Members of the FDA,

It is with great enthusiasm that Hope in Focus supports this application requesting Rare Pediatric Disease Designation for *NPHP5*-associated retinal dystrophies.

Hope in Focus is a non-profit patient advocacy organization for those living with Leber congenital amaurosis (LCA). LCA is a rare inherited retinal disease characterized by severe vision loss early in life. Most children with this disease are born with little or no vision, others may have significant vision loss in the first few years of life, stable vision for a period of time, and then eventually complete vision loss as the retina deteriorates into total blindness. Lastly, nearly all are legally blind before they enter elementary school.

Our mission is to generate awareness, raise funds for research, and provide outreach, support, and education to those affected by LCA and other rare retinal diseases. We know firsthand the need for support, hope, and research to treat individuals who are affected. We also work closely with the research and regulatory community for education and to advance treatments in a meaningful way for those who are impacted. Hope in Focus was co-founded by Laura Manfre and Charles Priebe after their daughter Sofia was diagnosed with LCA caused by a mutation in her *NPHP5* gene. While the organization has always supported all forms of LCA, we hold great hope for the prospect of a gene therapy to address *NPHP5*-associated retinal dystrophies.

In October 2023, we hosted a Patient Listening Session with the FDA. [redacted], living with LCA (*NPHP5*-LCA) shared [redacted] experience and the difficulties [redacted] faces with safety, independence, navigation, and socialization.

“The biggest thing is safety. Just in daily living, lack of sight poses a lot of risks, such as when traveling, cooking, and just getting around. Lighting changes, darkness, and other conditions lead to increased difficulty in my functional vision. I have difficulty navigating, especially outside of my home, and often just learning to do things is a big challenge for me. Another challenge relates to socialization because I can’t make observations easily and that impacts my ability to make connections and often leads to a lot of staring in public!”

[redacted] experience is not unique. We hear from other families and children about the challenges at school and relationships. Social isolation and safety are top concerns, and too many children and young adults living with this disease have relayed to us their challenges with depression, anxiety, self-harm, and other mental health issues resulting from the degradation of their vision.

There are currently no approved treatments or other clinical trials for *NPHP5*-associated retinal dystrophies.

We feel this program is the perfect candidate for the RPDD designation as it affects children under the age of 18, has a very serious impact on their physical, mental and social well-being, and is an ultra-rare disease affecting only around 5,000 patients worldwide.

Sincerely,

[redacted]

## Appendix 4:

### NPHP5 transgene expression in WT dogs treated with AAV-NPHP5

The purpose of this study was to verify in a single WT dog (animal ID: RC718) that subretinal injection of two titers (OD: 4.76 x $10^{12}$ vg/mL, and OS: 4.76 x $10^{11}$ vg /mL; SRI vol: 100 µL) of ssAAV2/5-GRK1-hNPHP5-WPRE led to expression of *hNPHP5* mRNA in photoreceptor cells. ISH was favored over immunohistochemistry due to the lack of reliable antibody directed against hNPHP5 protein that could be used on paraformaldehyde-fixed cryosections.

Retinas were collected 8 weeks PI, and ISH showed much stronger expression of the *hNPHP5* transgene in the eye (OD) injected with the higher titer. Labeling was found only in the treated/bleb area, confirming that the RNAscope probes are specific to human *NPHP5* and do not detect endogenous canine *NPHP5* mRNA (Fig. 1). When RNAscope probes that target canine *NPHP5* were used, a similar pattern of labeling was seen (Fig. 1). This suggests that canine probes are not specific, and can detect human NPHP5, but also, that the endogenous levels of canine *NPHP5* may be so low that they are not detectable by ISH. Taken together these results suggest that a titer of 4.76 x $10^{11}$ vg/mL may be high enough to lead to levels of transgene expression that are above physiological levels. No clinical signs of ocular toxicity nor inflammation were noted during the 8-week PI period (Fig. 2). Histological evaluation (by hematoxylin and eosin (H&E) staining) of the retinas was unremarkable.

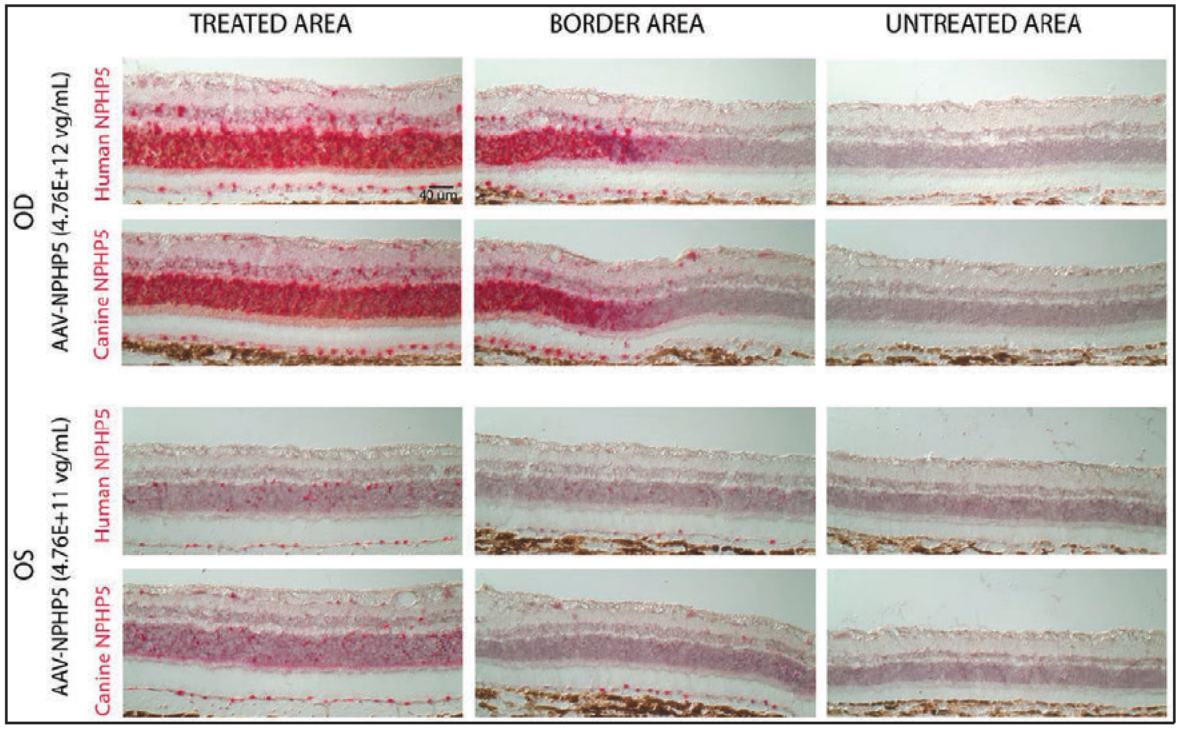


**Figure 1: RNA in situ hybridization for human NPHP5 transgene in the retinas of a WT dog (RC718) subretinally-injected with AAV-NPHP5 at two different titers.**

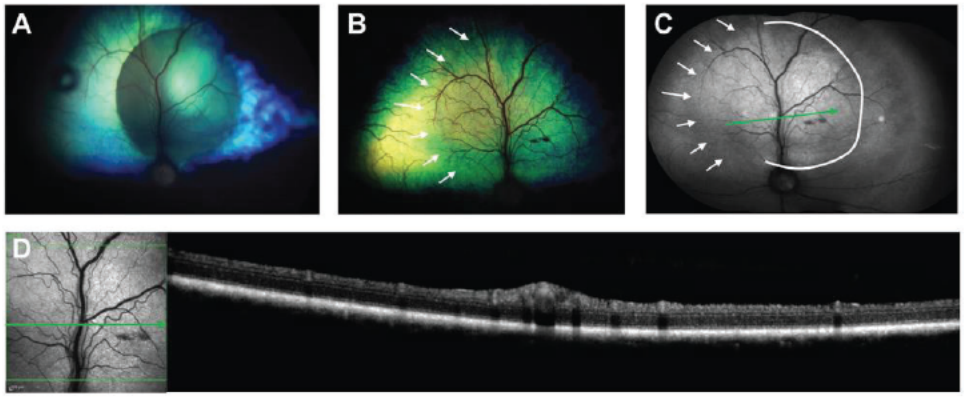


**Figure 2: In vivo retinal imaging of OD of WT dog (RC718) injected with AAV-NPHP5 at 4.76 x $10^{11}$ vg/mL. (A)** fundus photography immediately after formation of the subretinal bleb. **(B)** fundus photography at 8 wks PI (white arrows point to border of the treated/untreated area). **(C)** IR cSLO composite image at 8 wks PI. **(D)** cSLO/OCT at 8 wks PI.

## Appendix 5:

### Efficacy of AAV-NPHP5 in mutant NPHP5 dogs treated at early/mid-stage disease

The purpose of this study was to assess the long-term (multi-year) efficacy of a single uniocular subretinal injection of ssAAV2/5-GRK1-hNPHP5-WPRE (titer: 4.76 x $10^{11}$ vg/mL; SRI vol: 50 or 110 µL) in two *NPHP5* mutant dogs (animal IDs:AS2-447, WM66) treated at early/mid-stage disease (~ 8 weeks of age).

OCT retinal imaging showed a rescue effect on ONL thickness that was first clearly seen 24 weeks PI in both dogs and was still present 108 weeks PI in dog AS2-447 (Fig. 1) and at 87 weeks in dog WM66. There were no clinical signs of ocular toxicity nor inflammation noted during these time periods in either dog.

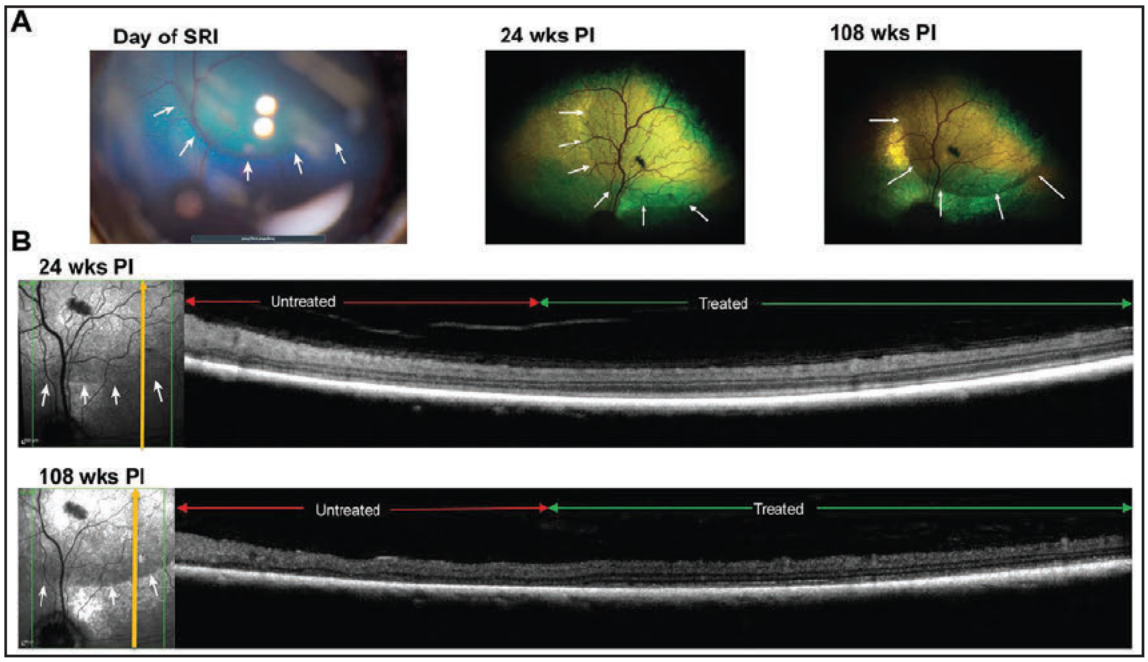


**Figure 1: Illustration of long-term structural rescue following subretinal injection of AAV-NPHP5 (110 µL at 4.76 x $10^{11}$ vg/mL) in a mutant dog (ID: AS2-447) treated at early/mid stage disease (8 wks of age).** (**A**) Color fundus photography immediately after formation of the subretinal bleb and at 24 and 48 wks post-injection. White arrows point towards the border of the treated area. Preserved reflectivity and retinal vasculature is seen within the treated area. (**B**) NIR cSLO and OCT (b scan) imaging of the border of the treated/untreated areas show retained ONL thickness and IS/OS signal at 24 wks PI that is still present at 108 wks PI.

ERG analysis showed in the subretinally-injected eye (Green traces) restoration of cone-mediated function as early as 4 weeks PI that increased in signal up to 24 weeks PI and remained present at 108 weeks PI in dog AS2-447 (Fig. 2) and 87 weeks PI in dog WM66. Rod-mediated function was improved as early as 4 weeks PI when it reached its highest amplitudes.

It reduced overtime (likely due to rod loss in untreated areas of the injected eye) but was still detectable at 108 weeks PI in dog AS2-447 but absent by 48 weeks PI in dog WM66.

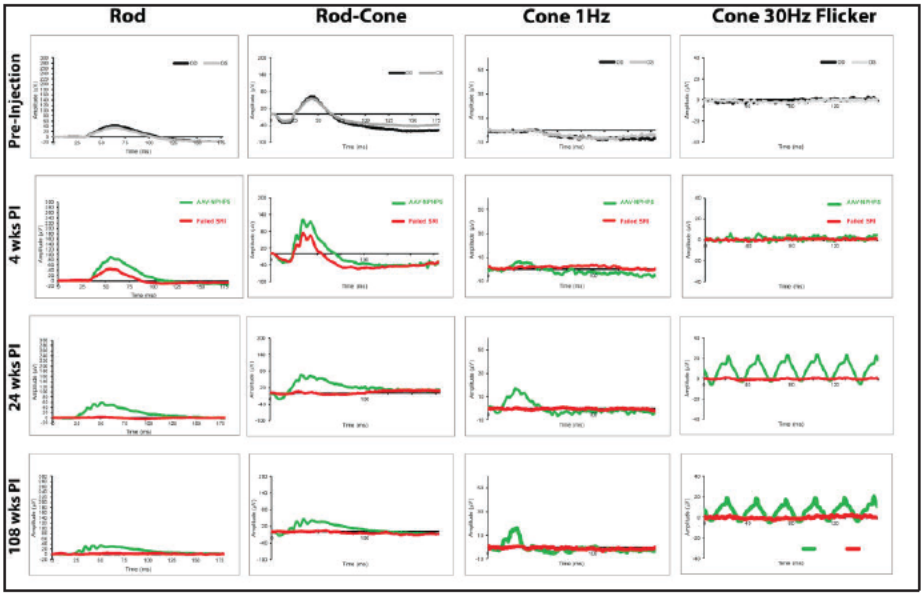


**Figure 2: Illustration of long-term rescue of rod function and restoration of cone function measured by electroretinography following subretinal injection of AAV-NPHP5 (110 µL at 4.76 x $10^{11}$ vg/mL) in the right eye (OD) of a mutant dog (ID: AS2-447) treated at early/mid stage disease (8 wks of age).**

Testing of visually-guided behavior showed under scotopic, mesopic, and photopic conditions improved performance from the subretinally-injected eye with reduced number of collisions as early as 20 weeks PI (AS2-447). This positive effect was sustained up to 116 weeks PI (Fig. 3).

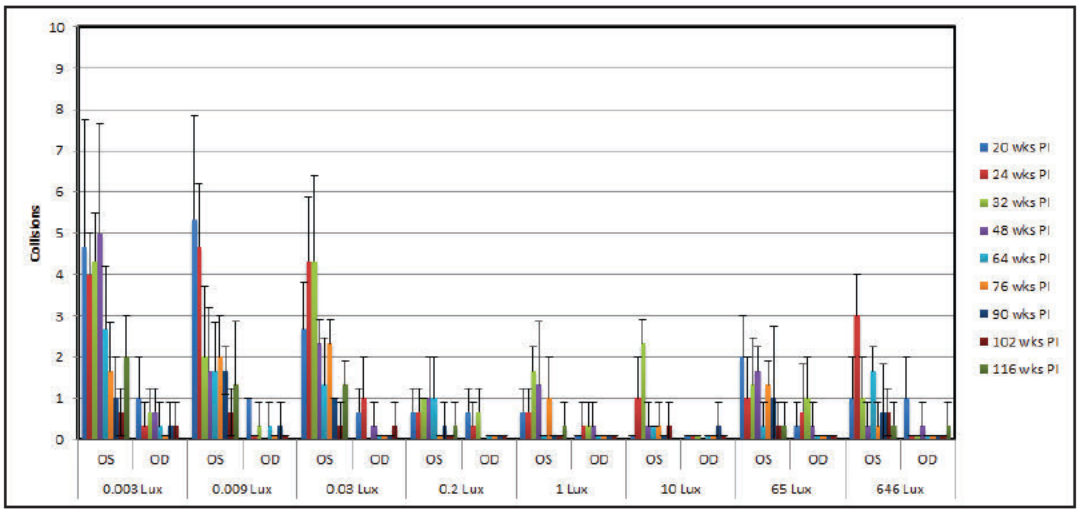


**Figure 3: Illustration of improved visual performance in a multi-luminance obstacle avoidance course following subretinal injection of AAV-NPHP5 (110 µL at 4.76 x $10^{11}$ vg/mL) in the right eye (OD) of a mutant dog (ID: AS2-447) treated at early/mid-stage disease (8 wks of age).** Number of collisions (mean±SD) when testing separately each eye under scotopic, mesopic and photopic conditions at timepoints (20 -116 wks) post-injection.

Testing of visually-guided behavior in dog WM66 revealed visual improvement (reduced number of collisions) in the injected versus the contralateral injected eye only under mesopic (0.2-10 Lux) and photopic (65 and 646 Lux) conditions as early as 12 weeks PI. This positive effect was sustained at 101 weeks PI. Absence of prominent and sustained rescue of rods (in comparison to dog AS2-447) may be explained by the lower dose/ smaller bleb (50 µL) injected in this animal.

Taken together these results show long-term (> 2 years) structural and functional efficacy of this viral vector construct when delivered at early/mid stage-disease. This study is ongoing and to date no clinical signs of toxicity/inflammation have been observed.

## Appendix 6:

### Long term efficacy of AAV-NPHP5 in a mutant NPHP5 dog treated at late-stage disease

The purpose of this study was to assess the efficacy of a single uniocular subretinal injection of ssAAV2/5-GRK1-hNPHP5-WPRE (titer: 4.76 x $10^{11}$ vg/mL; SRI vol: 150 µL) in a *NPHP5* mutant dog (animal ID: AS2-448) at late-stage disease (24 weeks of age).

OCT retinal imaging showed a mild rescue effect on ONL thickness that was first clearly seen 48 weeks PI and was still detectable 97 weeks PI (Fig. 1). There were no clinical signs of ocular toxicity nor inflammation noted during this period.

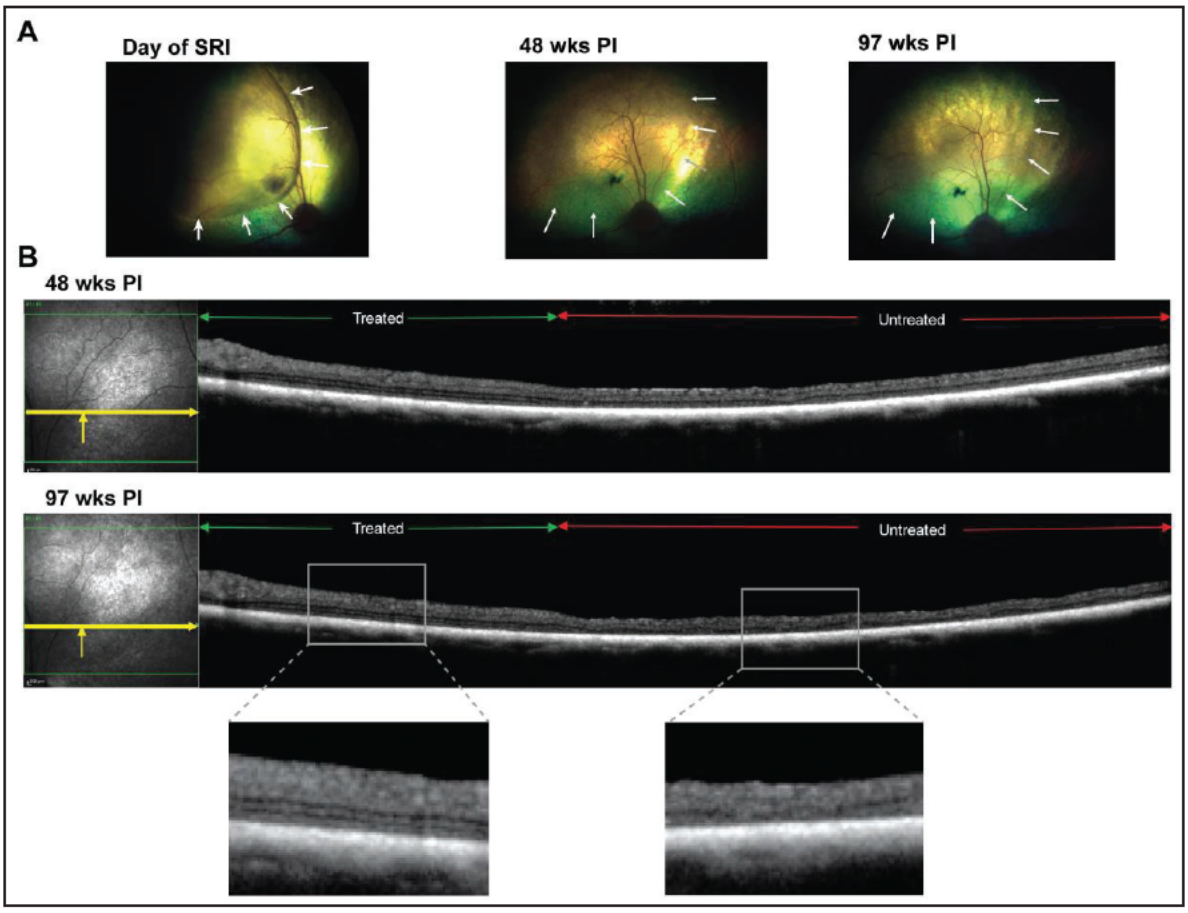


**Figure 1**: **Long-term structural rescue following subretinal injection of AAV-NPHP5 (150 µL at 4.76 x $10^{11}$ vg/mL) in a mutant dog (ID: AS2-448) treated at late-stage disease (24 wks of age).** (**A**) Color fundus photography immediately after formation of the subretinal bleb and at 48 and 97 wks post-injection. White arrows point towards the border of the treated area. Although increased reflectivity is seen, retinal vasculature appears preserved within the treated area. (**B**) NIR cSLO and OCT (b scan) imaging of the border of the treated/untreated areas show a thinned yet retained ONL and IS/OS signal at 48 wks PI that are still present at 97 wks PI.

ERG analysis (Fig. 2) showed in the injected eye (OD; green traces) restoration of cone-mediated function as early as 4 weeks PI that increased in signal up to 24 weeks PI and was still detectable at 97 weeks PI. Rod-mediated function was restored as early as 8 weeks PI and reached its highest amplitude by 12 weeks PI. After that, it decreased significantly but was still detectable at 97 weeks PI.

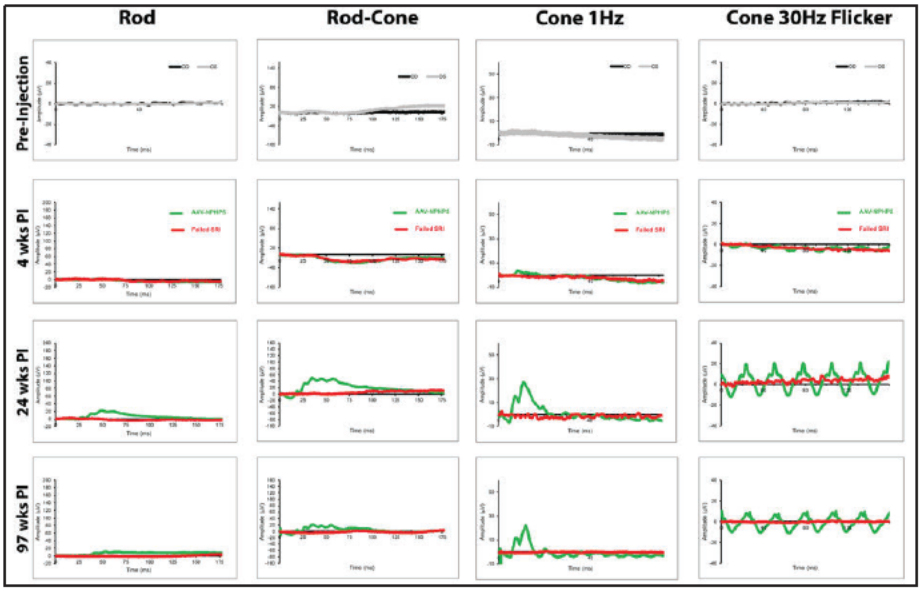


**Figure 2: Long-term restoration of rod and cone function measured by electroretinography following subretinal injection of AAV-NPHP5 (150 µL at 4.76 x $10^{11}$ vg/mL) in the right eye (OD) of a mutant dog (ID: AS2-448) treated at late-stage disease (24 wks of age).**

Testing of visually-guided behavior showed under scotopic, mesopic, and photopic conditions improved performance from the injected eye (OD) with reduced transit time and reduced number of collisions (Fig. 3) as early as 8 weeks PI. This positive effect was sustained at 102 weeks PI.

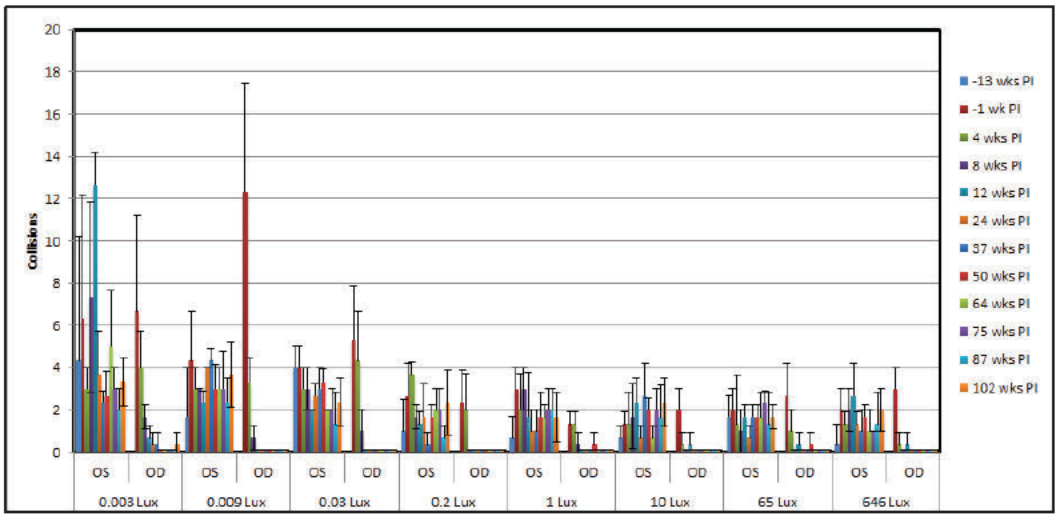


**Figure 3: Improved visual performance in a multi-luminance obstacle avoidance course following subretinal injection of AAV-NPHP5 (150 µL at 4.76 x $10^{11}$ vg/mL) in the right eye (OD) of a mutant dog (ID: AS2-448) treated at late-stage disease (24 wks of age).** Number of collisions (mean±SD) when testing separately each eye under scotopic, mesopic and photopic conditions at several timepoints (ranging from 13 wks before injection to 102 wks post-injection.

Taken together these results show long-term (~ 2 years) structural and functional efficacy of this viral vector construct when delivered at late-stage disease. Study is ongoing and to this date no clinical signs of toxicity/inflammation have been observed.

## Appendix 7:

### Therapeutic range and long-term stability of AAV-NPHP5 in mutant NPHP5 dogs

This study was conducted to determine the therapeutic range of ssAAV2/5-GRK1-hNPHP5-WPRE by assessing efficacy and safety in 3 NPHP5 mutant dogs injected uniocularly at mid-stage disease (~ 12 weeks of age) with one of three titers (4.76 $x10^{10}$; 1.0 x $10^{11}$; 4.76 x $10^{11}$ vg/mL). The overall goal being to identify the lowest dose that still provided some efficacy.

In the dog (animal ID WM62) that was treated with the lowest dose (4.76 $x10^{10}$ vg/mL; SRI vol: 150 µL) funduscopic examination revealed increased reflectivity and attenuation of the retinal vasculature even in the treated area, yet, OCT retinal imaging showed as early as 12 weeks post-injection (PI) some very faint rescue of ONL thickness that was limited to the visual streak region within the much larger treated/bleb area. This limited rescue effect was still detectable at 96 weeks PI (Fig. 1A). There were no clinical signs of ocular toxicity nor inflammation noted during this period.

In the dog (animal ID WM63) that was treated with the mid dose (1.0 $x10^{11}$ vg/mL; SRI vol: 150 µL) funduscopic examination revealed increased reflectivity yet retinal vasculature was better preserved in the treated area. In addition, OCT retinal imaging showed by 47 weeks PI some faint rescue of ONL thickness that was limited to the visual streak and immediate dorsal region within the much larger treated/bleb area. This limited rescue effect was still detectable at 96 weeks PI (Fig. 1B). There were no clinical signs of ocular toxicity nor inflammation noted during this period.

In the dog (animal ID WM67) that was treated with the highest dose (4.76 $x10^{11}$ vg/mL; SRI vol: 70 µL) funduscopic examination revealed some mild increased reflectivity in the treated area that was less pronounced than in surrounding untreated regions and retinal vasculature was better preserved in the treated area. In addition, OCT retinal imaging showed a slight loss of ONL thickness within the first 12 weeks PI, after which ONL thickness appeared to stabilize. Faint rescue of ONL thickness in the treated area versus untreated area of the same eye was first detectable by 24 weeks, was more obvious at later points, and still persisted at 97 weeks PI (Fig. 1C). There were no clinical signs of ocular toxicity nor inflammation noted during this period.

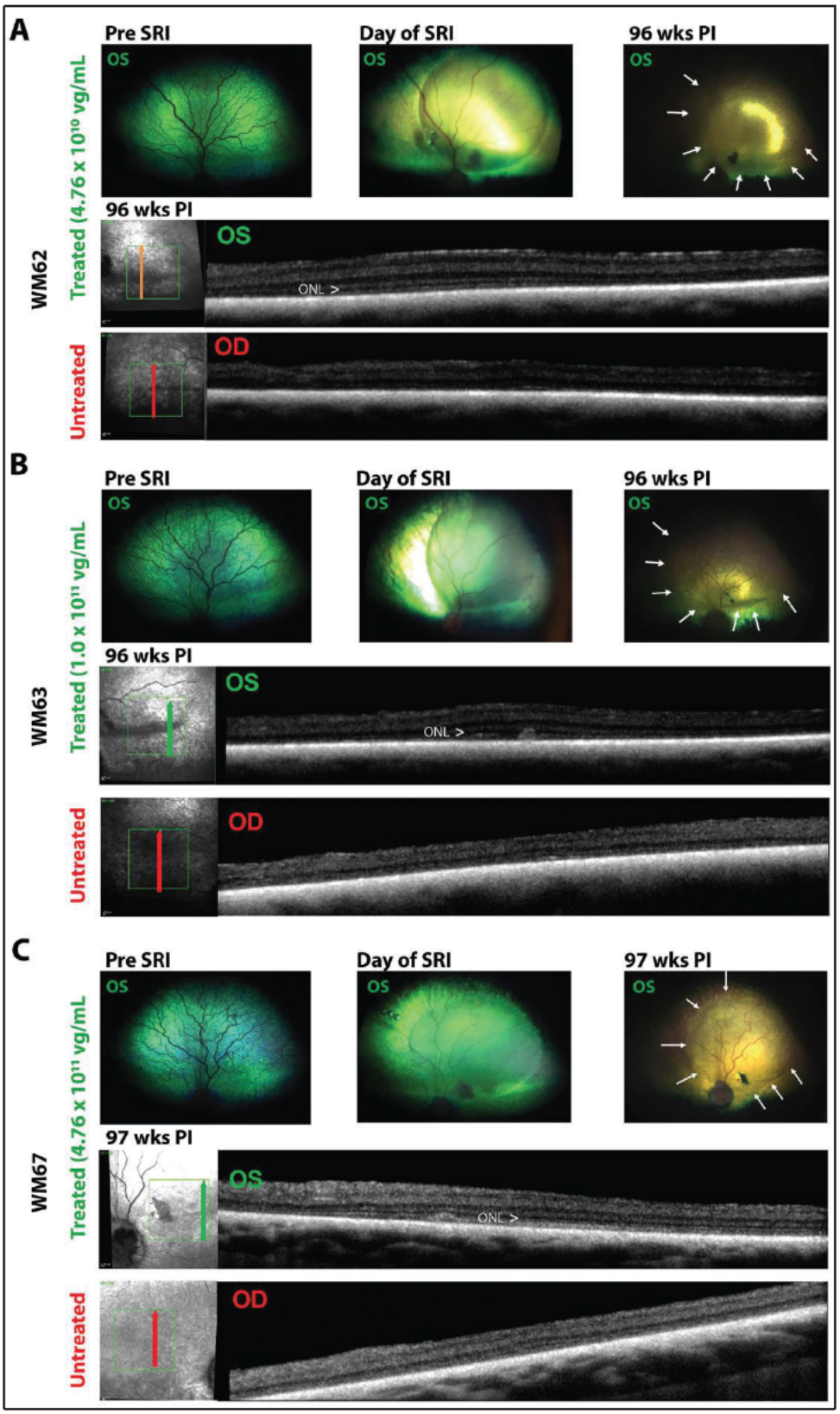


**Figure 1**: Fundus photography and cSLO/OCT imaging of NPHP5 dogs treated with 3 different titers of AAV-NPHP5.

(**A**) WM62 dog subretinally-injected in OS with 150 µL at 4.76 x$10^{10}$ vg/mL.

(**B**) WM63 dog subretinally-injected in OS with 150 µL at 1.0 x$10^{11}$ vg/mL.

(**C**) WM67 dog subretinally-injected in OS with 70 µL at 4.76 x$10^{11}$ vg/mL.

ERG analysis of dog WM62 (treated with the low dose) did not show in the injected eye any restoration of rod function. Mild restoration of cone-mediated function was first seen at 12 weeks PI, was still detectable at 60 weeks PI, yet barely detectable at 84 weeks PI (Fig. 2).

ERG analysis of dog WM63 (treated with the mid dose) did not show in the injected eye any restoration of rod function. Restoration of cone-mediated function was first seen at 8 weeks PI, and although decreased, was still detectable at 84 weeks PI (Fig. 2).

ERG analysis of dog WM67 (treated with the high dose) showed in the injected eye that rod-mediated function was restored as early as 8 weeks PI, reached its highest amplitudes by 12 weeks PI, and although decreased was still detectable at 84 weeks PI. Pronounced restoration of cone-mediated function was seen as early as 4 weeks PI, increased in amplitude up to 12 weeks PI and remained stable and detectable at 84 weeks PI (Fig. 2).

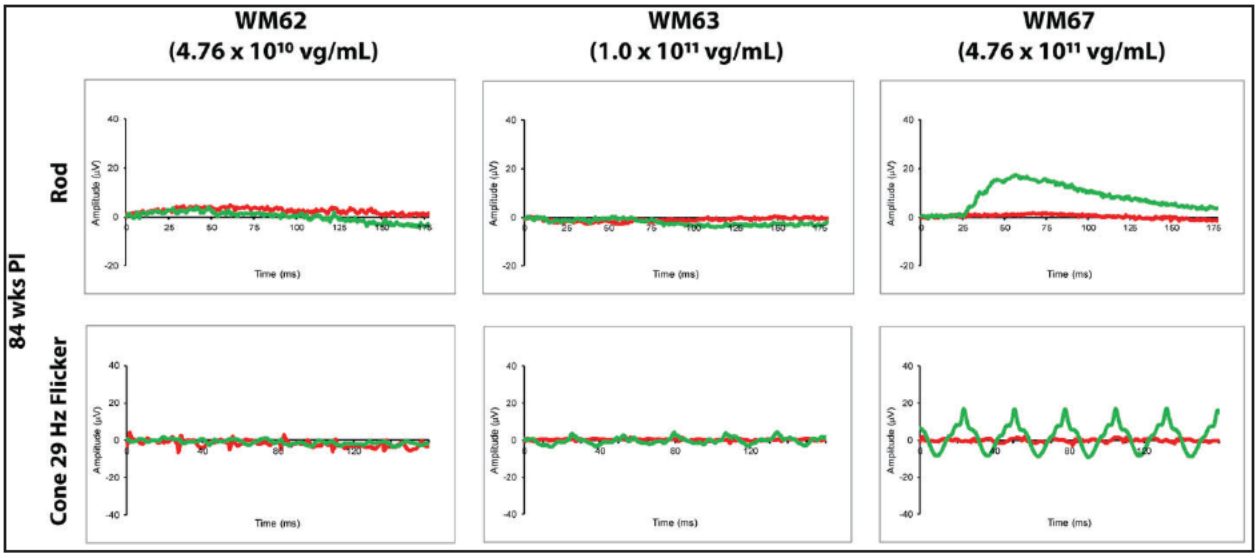


**Figure 2: Rod and Cone-mediated ERG traces at the latest timepoint investigated (84 wks PI) in mutant NPHP5 dogs injected in one eye (OS, green traces) with different titers of AAV-NPHP5.**

Testing of visually-guided behavior of dog WM62 (low dose) showed under photopic (10 to 646 Lux) improved performance from the injected eye (OS) with reduced number of collisions as early as 8 weeks PI. This positive effect was sustained at 90 weeks PI (Fig. 3).

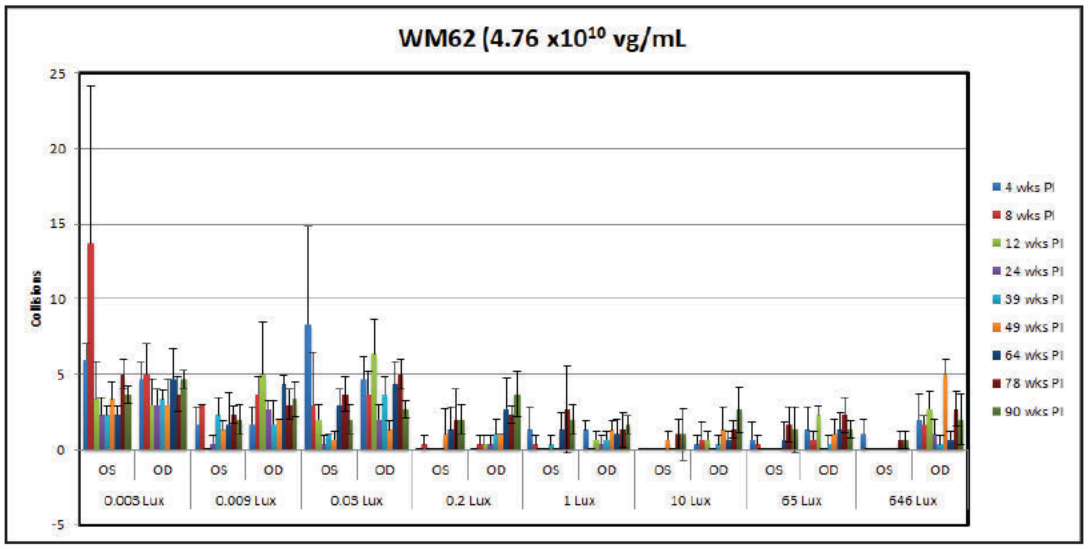


**Figure 3: Illustration of improved visual performance under photopic conditions in a multi-luminance obstacle avoidance course following subretinal injection of AAV-NPHP5 (150 µL at 4.76 x $10^{10}$ vg/mL) in the left eye (OS) of a mutant dog (ID: WM62) treated at mid-stage disease (12 wks of age).** Number of collisions (mean±SD) when testing separately each eye under scotopic, mesopic and photopic conditions at timepoints (4 -90 wks) post-injection.

Testing of visually-guided behavior of dog WM63 showed under mesopic and photopic conditions improved performance from the injected eye (OS) with reduced transit time and reduced number of collisions as early as 8 weeks PI. This positive effect was sustained at 93 weeks PI (Fig. 4).

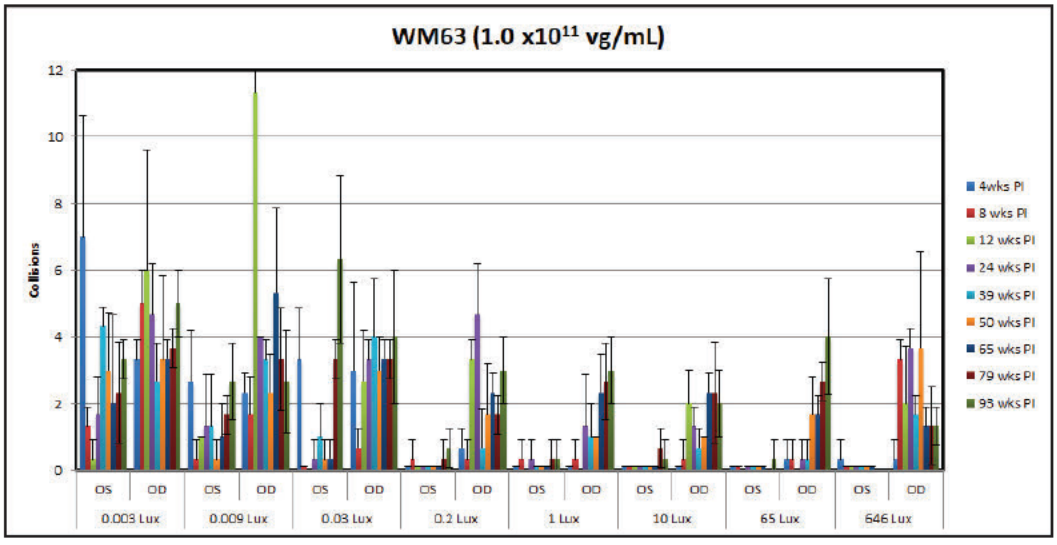


**Figure 4: Illustration of improved visual performance under photopic, mesopic and photopic conditions in a multi-luminance obstacle avoidance course following subretinal injection of AAV-NPHP5 (150 µL at 1.0 x $10^{11}$ vg/mL) in the left eye (OS) of a mutant dog (ID: WM63) treated at mid-stage disease (12 wks of age).** Number of collisions (mean±SD) when testing separately each eye under scotopic, mesopic and photopic conditions at timepoints (4 -93 wks) post-injection.

Testing of visually-guided behavior of dog WM67 showed under scotopic, mesopic, and photopic conditions improved performance from the injected eye (OS) with reduced transit time and reduced number of collisions as early as 8 weeks PI. This positive effect was sustained at 93 weeks PI (Fig. 5).

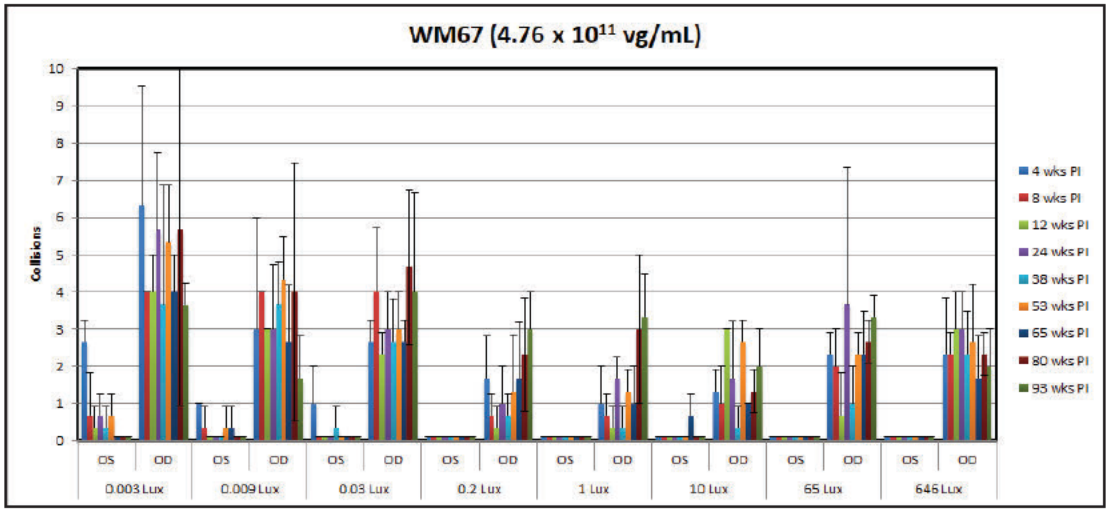


**Figure 5: Illustration of improved visual performance under photopic, mesopic and photopic conditions in a multi-luminance obstacle avoidance course following subretinal injection of AAV-NPHP5 (70 µL at 4.76 x $10^{11}$ vg/mL) in the left eye (OS) of a mutant dog (ID: WM67) treated at mid-stage disease (12 wks of age).** Number of collisions (mean±SD) when testing separately each eye under scotopic, mesopic and photopic conditions at timepoints (4 -93 wks) post-injection.

Taken together these results show limited yet detectable restoration of cone-mediated function at the lowest tested titer (4.76 x $10^{10}$ vg/mL). Restoration of rod-mediated function was only detected with a log higher titer (4.76 x $10^{11}$ vg/mL).