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

Adeno-Associated Virus 5 targeting the human Cyclic Nucleotide Gated Channel Subunit Beta 1 (CNGB1) gene (AAV5-CNGB1) for the Treatment of Autosomal Recessive Retinitis Pigmentosa (ARRP) due to CNGB1 mutations

Rare Pediatric Disease Designation Request

June 27, 2024

- CONFIDENTIAL -

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LIST OF ABBREVIATIONS

NIH - National Institutes of Health
NCATS - National Center for Advancing Translational Sciences
AAV - Adeno-Associated Virus
AAV5-CNGB1 - Adeno-Associated Virus 5 vector expressing a functional human Cyclic Nucleotide Gated Channel Subunit Beta 1 gene
ARRP - Autosomal Recessive Retinitis Pigmentosa
CNGB1 - Cyclic Nucleotide Gated Channel Subunit Beta 1
RHO - Rhodopsin
ERG - Electroretinography
NAC - N-Acetylcysteine
FDA - Food and Drug Administration
ODDR - Orphan Drug Designation Request
SD-OCT - Spectral-Domain Optical Coherence Tomography
ELM - External Limiting Membrane
EZ - Ellipsoid Zone
IZ - Interdigitation Zone
GARP - Glutamic Acid Rich Protein
CNG - Cyclic Nucleotide-Gated
cGMP - Cyclic Guanosine Monophosphate
PDE6 - Phosphodiesterase 6

1 RARE PEDIATRIC DISEASE DESIGNATION REQUEST STATEMENT

Pursuant to 21 CFR 316.20, National Institutes of Health (NIH), National Center for Advancing Translational Sciences (NCATS) respectfully requests that the recombinant adeno-associated virus vector 5 targeting the human Cyclic Nucleotide Gated Channel Subunit Beta 1 (CNGB1) for use in the treatment of Autosomal Recessive Retinitis Pigmentosa (ARRP) receive designation as a rare pediatric disease. Retinitis pigmentosa is an inherited retinal dystrophy caused by mutations in the CNGB1 gene which leads to the death of rod photoreceptors, followed by the gradual loss of cone photoreceptors and progressive visual loss.

2 ADMINISTRATIVE INFORMATION

2.1 Sponsor

National Institutes of Health 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 Contacts

Regulatory Correspondent

[redacted]

Sponsor Contact

[redacted]

Principal Investigator

[redacted]

2.3 Drug Name

Chemical Name - Drug Substance

Adeno-Associated Virus 5 vector expressing a functional human Cyclic Nucleotide Gated Channel Subunit Beta 1 (CNGB1) gene (AAV5-CNGB1)

Generic/Trade Name - Drug Product

Generic: A recombinant Adeno-Associated Virus 5 vector expressing a functional human Cyclic Nucleotide Gated Channel Subunit Beta 1 (CNGB1) gene (AAV5 -CNGB1), under control of a short human rhodopsin (RHO) promoter

Trade Name: Not yet available.

2.4 Manufacturer's Name and Address

Drug Substance Manufacturer

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

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

Autosomal recessive retinitis pigmentosa due to CNGB1 mutations is a rare genetic disorder characterized by the degeneration of photoreceptors in the retina, leading to severe visual impairment and eventually blindness.[1] The disease typically manifests in early childhood and progresses gradually.

Molecular Etiology and Genetics

Autosomal recessive retinitis pigmentosa due to CNGB1 mutations is caused by biallelic mutations in the CNGB1 gene, which encodes the beta-subunit of the rod cyclic nucleotide-gated channel.[2,3] This channel is essential for the conversion of light signals into electrical signals in

the rod photoreceptors of the retina. Loss-of-function mutations in CNGB1 disrupt this process, leading to progressive degeneration of rod photoreceptors, and secondary loss of cone function and vision.[4]

Clinical Manifestations and Symptoms

The disease typically presents in childhood or early adolescence. Initial symptoms include night blindness and loss of peripheral vision, progressing to tunnel vision and eventually leading to complete blindness. The visual impairment is often accompanied by the appearance of bone-spicule pigmentation in the retina, a characteristic sign observed during ophthalmic examination.

Epidemiology

The precise prevalence of autosomal recessive retinitis pigmentosa due to CNGB1 mutations is not well-documented, but it is estimated to represent about 2-4% of all retinitis pigmentosa cases.[5-7] Given the rarity and genetic basis, the condition is significantly underdiagnosed, with a need for greater awareness and genetic screening.

Pathophysiology

In the absence of functional CNGB1 protein, there is a failure in the phototransduction cascade, which is critical for the normal functioning of rod photoreceptors. Initially, this leads to impaired dark adaptation and progresses to a complete loss of rod function. Subsequently, the degeneration of rods places stress on the cone photoreceptors, which also begin to degenerate, leading to a further decline in visual acuity and color perception.[8-11]

Natural History

The progression of the disease is slow but relentless, with most affected individuals experiencing legal blindness by the third to fourth decade of life. The gradual loss of vision significantly impacts the quality of life, limiting educational, social, and employment opportunities for affected individuals.[10,12,13]

Current Diagnostic and Treatment Landscape

Diagnosis is typically based on clinical examination, family history, electroretinography (ERG), and confirmed through genetic testing.[2,14] There is no cure for the disease, and treatment options are limited to managing symptoms and slowing progression, such as using N-Acetylcysteine (NAC) supplementation, which has shown some efficacy in slowing retinal degeneration. The advent of gene therapy presents a potential transformative approach to directly address the underlying genetic cause of the disease.

3.2 Proposed Indication and Use of AAV5-CNGB1

Treatment involves subretinal injection of AAV5-CNGB1, aimed at delivering a normal copy of the CNGB1 gene directly to retinal cells, thereby potentially halting or reversing the photoreceptor degeneration process.

Proposed Indication: The AAV5-CNGB1 gene therapy is indicated for the treatment of autosomal recessive retinitis pigmentosa specifically due to biallelic mutations in the CNGB1 gene. This genetic therapy aims to provide a functional copy of the CNGB1 gene to retinal cells, thereby restoring the normal phototransduction process and preventing further degeneration of the photoreceptors.

Mechanism of Action: AAV5-CNGB1 utilizes an adeno-associated virus serotype 5 (AAV5) as a vector to deliver a normal copy of the CNGB1 gene directly into the retinal cells. Once administered via a subretinal injection, the viral vector transduces the photoreceptor cells, particularly the rods, where it integrates into the host cell genome and expresses the CNGB1 protein.[2,15-19] This expression is expected to restore the function of the cyclic nucleotide-gated channels disrupted by the mutations, thereby preserving the structure and function of the retina's photoreceptor cells.[16]

Route of Administration: The gene therapy is administered through a subretinal injection, which involves a surgical procedure where the vector is injected directly under the retina in the affected eye. This route is chosen to target the photoreceptor cells effectively and to maximize the local concentration of the vector while minimizing systemic exposure.

Rationale for Use in the Indicated Population: Patients with autosomal recessive retinitis pigmentosa due to CNGB1 mutations typically experience progressive vision loss beginning in childhood, with few if any, treatment options available that address the underlying cause of the disease. The use of AAV5-CNGB1 gene therapy is particularly compelling for this patient population as it offers a potentially curative treatment by correcting the genetic defect that leads to photoreceptor degeneration.

Clinical Development and Expected Benefits: The clinical development of AAV5-CNGB1 is supported by robust preclinical studies demonstrating that the therapy can effectively deliver the CNGB1 gene and restore photoreceptor function in animal models.[17,18] Early-phase clinical trials aim to evaluate the safety and dosage of this therapy, with subsequent studies focused on assessing its efficacy. The primary benefit expected from AAV5-CNGB1 therapy is the stabilization or improvement in visual function, which could significantly enhance the quality of life and independence of affected individuals.[17,18]

Integration into Current Treatment Paradigms: Given that there are no effective treatments available that halt the progression of retinitis pigmentosa due to CNGB1 mutations, AAV5-

CNGB1 does not compete with existing therapies but rather provides a novel treatment modality. If successful, this therapy could set a new standard of care for this form of retinitis pigmentosa, transforming the therapeutic landscape for a condition currently marked by inevitable progression to blindness.

3.3 Reasons Why Such Therapy Is Needed

Currently, there are no effective treatments available for this form of retinitis pigmentosa, and the proposed gene therapy could offer a significant improvement in the quality of life and visual function for affected children.

Lack of Effective Treatments: Currently, there are no effective therapies available that can halt the progression or reverse the symptoms of autosomal recessive retinitis pigmentosa due to CNGB1 mutations. Existing management strategies are primarily supportive and aim to maximize remaining vision through the use of visual aids and lifestyle adjustments. These strategies do not address the underlying genetic cause of the disease.

Progressive and Debilitating Nature of the Disease: Autosomal recessive retinitis pigmentosa is a progressive disorder that typically leads to severe visual impairment and ultimately blindness. The progression from night blindness to complete vision loss profoundly impacts the quality of life, affecting educational opportunities, employability, and social interactions. The inevitable decline in visual function creates a substantial burden for patients and their families, both psychologically and economically.

Potential for Restoration and Preservation of Vision: Gene therapy offers a transformative approach by potentially restoring visual function or at least significantly slowing the progression of the disease. AAV5-CNGB1 is designed to deliver a normal copy of the CNGB1 gene directly to the affected retinal cells, potentially restoring the normal function of these cells and preserving vision.[17,18] Successful gene therapy could reduce the overall burden of disease and improve the life trajectory of affected individuals.

Pediatric Population: Since symptoms of the disease typically begin in childhood, early intervention is crucial to prevent irreversible damage to the retina. The pediatric population stands to benefit significantly from a therapy that can preserve or restore vision during the formative years, greatly impacting their developmental and educational outcomes.

Safety and Targeted Delivery: The use of AAV vectors for gene delivery has been well-studied and has shown a favorable safety profile in other ocular gene therapy trials. Subretinal administration of AAV5-CNGB1 ensures that the therapy is delivered directly to the target tissue, which maximizes its efficacy while minimizing potential systemic effects. This targeted approach is crucial for treating localized genetic disorders such as retinitis pigmentosa. [17,18]
Innovative Approach with Broad Implications:

Developing effective gene therapies for rare genetic disorders not only benefits patients with autosomal recessive retinitis pigmentosa but also enhances the broader field of genetic medicine.

Success with AAV5-CNGB1 could pave the way for similar therapies for other genetic conditions, expanding the impact of this innovative treatment approach.

4 DESCRIPTION OF AAV5-CNGB1 AND SCIENTIFIC RATIONALE FOR USE

4.1 Description of AAV5-CNGB1

4.1.1 Drug Product Naming Convention

AAV5-CNGB1

4.1.2 Drug Product Physical, Chemical, and Pharmaceutical Properties

Overview of AAV5-CNGB1 Construct

The complete nucleotide sequence of AAV5-CNGB1 is included as Appendix A. The construct comprises several key elements designed to ensure efficient gene delivery and expression within the target cells (**Table 1**). All sequence manipulations were verified by sequencing.

Table 1. Overview of AAV5-CNGB1 Construct

Element	Description
Inverted Terminal Repeats (ITRs)	Length: 131 bp each, originating from the pSub201 cis-plasmid, flanking the transgene cassette for packaging and integration.
Enhancer	Not included.
Promoter	Short human rhodopsin (hRHO) promoter, 194 bp, driving rod-specific gene expression, PCR amplified from the full-length hRHO promoter.
Intron	Not included.
5' Untranslated Region (UTR)	Not included.
Therapeutic Gene	Full-length transcript variant 1 of human CNGB1 (NM_001297.4; 3.75 kb), encoding the beta subunit of the rod CNG channel, PCR amplified.
3' Untranslated Region (UTR)	Not included.
Polyadenylation Signal	Short polyadenylation signal, 221 bp, acting as an effective termination and polyadenylation enhancer.
3' Inverted Terminal Repeat	Length: 131 bp.
Genome Configuration	Single-stranded DNA, designed for efficient packaging and expression within retinal cells.
Remarks	Includes regulatory sequences such as randomized synthetic DNA fragments. Contains a pUC18 origin of replication and a kanamycin resistance gene (KanR).

The vector uses the AAV5 serotype, which has a strong affinity for retinal tissue, ensuring that the delivered gene reaches the intended photoreceptor cells. The human CNGB1 gene included in the vector is optimized for maximal expression and functional compatibility within human retinal cells.

Impact on Expression

The short human rhodopsin (hRHO) promoter is selected for its high efficiency in driving rod-specific gene expression, ensuring that the therapeutic gene is expressed predominantly in rod photoreceptors. The choice of a single-stranded genome configuration facilitates efficient packaging within the AAV5 capsid and supports stable gene expression within the target cells.

Schematic of the Plasmid Map

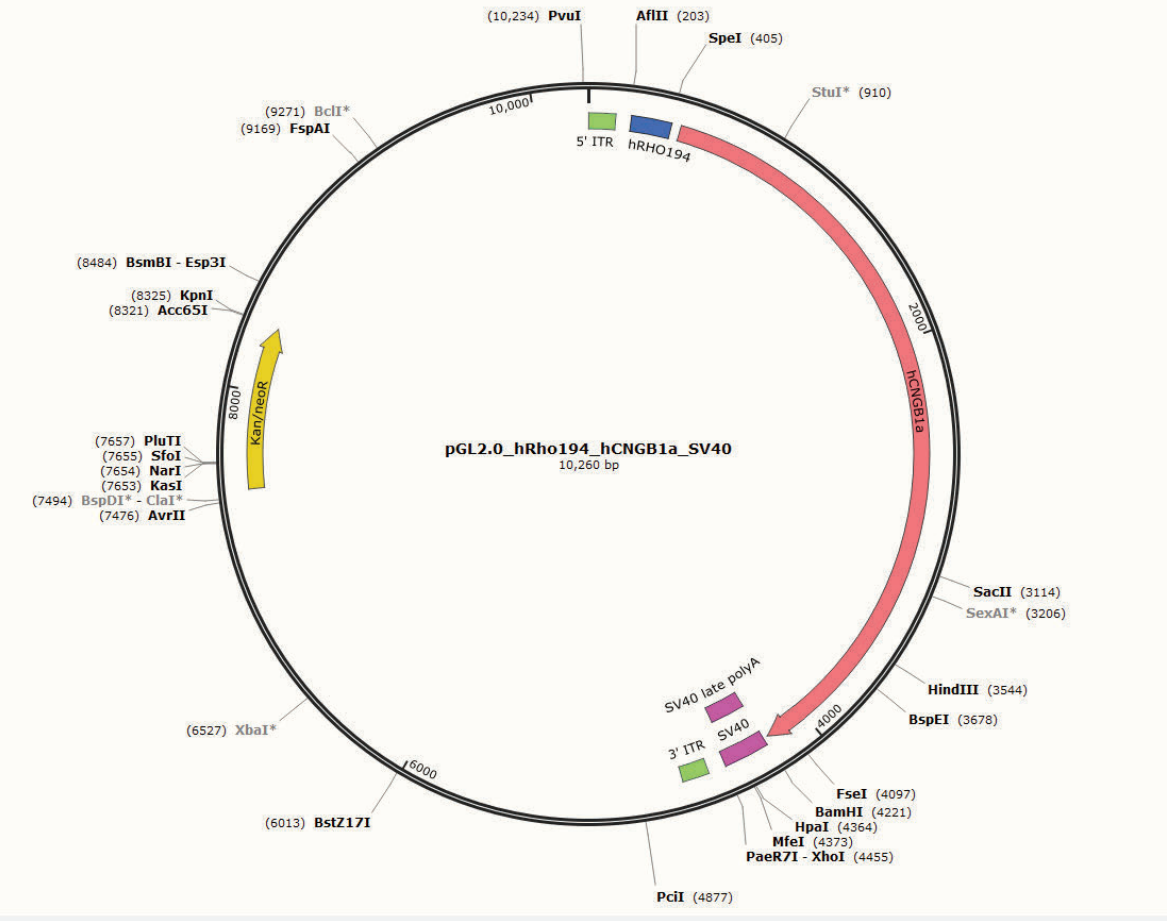
The following schematic represents the plasmid map used to produce the therapeutic transgene. Major components of the transgene, as well as other features such as antibiotic resistance, origin of replication, and details of the plasmid backbone, are labeled. The size of the transgene and its constitutive elements are also denoted in **Fig. 1, Fig. 2**. See **Appendix A** for the transgene nucleotide sequence.

Figure 1. Promoter Sequence

Sequence of the hRHO194 promoter

1	TCTCCTCCCT	GACCTCAGGC	TTCCTCCTAG	TGTCACCTTG	GCCCCTCTTA	GAAGCCAATT
61	AGGCCCTCAG	TTTCTGCAGC	GGGGATTAAT	ATGATTATGA	ACACCCCCAA	TCTCCCAGAT
121	GCTGATTCAG	CCAGGAGCTT	AGGAGGGGGA	GGTCACTTTA	TAAGGGTCTG	GGGGGGTCAG
181	AACCCAGAGT	CATC				

Figure 2. Plasmid Construct Diagram



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

Drug Product Description: AAV5-CNGB1 is a gene therapy product consisting of a recombinant adeno-associated virus type 5 (AAV5), which is used as a vector to deliver the CNGB1 gene.[17,18] The vector contains a normal, human sequence of the CNGB1 gene under the control of a promoter effective in photoreceptor cells. The therapeutic product is manufactured as a sterile liquid for subretinal injection, prepared in a phosphate-buffered saline solution with additional excipients to stabilize the virus during storage and administration.

Formulation Details: The formulation is designed to be isotonic, with a pH optimized for ocular administration, reducing the risk of irritation or tissue damage upon injection. It contains buffers to maintain pH, salts to manage osmolarity, and sugars as cryoprotectants to support long-term storage at ultra-low temperatures.

Mechanism of Action: The primary mechanism by which AAV5-CNGB1 treats retinitis pigmentosa is through gene replacement therapy. Following subretinal injection, the AAV5 vector transduces photoreceptor cells in the retina. Once inside the cells, the vector releases its genetic payload, which includes a functional copy of the CNGB1 gene. This gene is then transcribed and translated into the CNGB1 protein, a crucial component of the rod photoreceptors' light-sensing

machinery. Restoring CNGB1 function in these cells is expected to halt the progression of photoreceptor degeneration, preserving vision. [17,18] The CNGB1 protein expressed by the therapy helps to reestablish the normal phototransduction cascade that converts light into electrical signals in the retina.8-11 This restoration is anticipated to stabilize or possibly improve the visual function in affected individuals, significantly altering the disease's natural progression.

Route of Administration: AAV5-CNGB1 is administered via a subretinal injection, which is a surgical procedure performed under local anesthesia. The procedure involves creating a small retinotomy through which the vector solution is injected directly under the retina. This approach targets the therapy directly to the affected area, maximizing the potential for the vector to reach the photoreceptor cells while minimizing systemic exposure.[16]

Rationale for Subretinal Delivery: Subretinal delivery is chosen because it allows the vector to be placed in close proximity to the photoreceptors, the primary cells affected in retinitis pigmentosa. This method enhances transduction efficiency and localizes the therapy's action to the retina, reducing the potential for off-target effects.[16] Additionally, delivering the therapy directly to the retina circumvents the blood-retinal barrier, which can limit the effectiveness of systemic treatments.

4.2 Scientific Rationale for the Use of AAV5-CNGB1

Preclinical studies in animal models have shown that AAV5-CNGB1 can effectively deliver the CNGB1 gene to the target cells, leading to restored photoreceptor function and preserved retinal structure.

Understanding the Genetic Pathology: Autosomal recessive retinitis pigmentosa due to mutations in the CNGB1 gene leads to a loss of function in the beta subunit of the rod photoreceptor cyclic nucleotide-gated (CNG) channel. This channel is essential for the phototransduction process that converts light into electrical signals in the retina. The absence or malfunctioning of CNGB1 disrupts this process, resulting in the progressive degeneration of rod photoreceptors, followed by the degeneration of cone photoreceptors, and ultimately leading to blindness. The CNGB1 gene therapy aims to restore the function of this critical protein by delivering a correct copy of the gene directly to the cells that require it.[12,13,15,20-29]

Preclinical Evidence Supporting Gene Therapy: Extensive preclinical studies in animal models of retinitis pigmentosa with CNGB1 mutations have shown promising results.[2,15-19] These studies have demonstrated that subretinal delivery of AAV5-CNGB1 can result in the expression of CNGB1 in rod photoreceptors, restoration of the phototransduction cascade, and preservation of retinal structure and function.[17,18] Electrophysiological assessments in these models have confirmed the restoration of retinal response to light stimuli, indicating functional recovery of photoreceptors.

Rationale for Subretinal Delivery of AAV5-CNGB1: The choice of AAV5 as a vector for delivering CNGB1 is based on its proven efficiency in transducing photoreceptors and its established safety profile in previous ocular gene therapy trials.[17,18] AAV vectors are generally well-tolerated and have shown long-term expression of therapeutic genes, which is crucial for

treating a chronic condition such as retinitis pigmentosa. The subretinal route ensures that the vector is delivered directly to the layer of the retina where rod photoreceptors are located, maximizing the therapy's efficacy and minimizing potential systemic exposure.[16]

Potential Clinical Impact: The introduction of a functional CNGB1 gene into the retina is expected to halt or significantly slow the progression of retinitis pigmentosa in affected individuals. This could preserve remaining vision longer than would naturally occur, potentially preventing blindness for years or even decades. For pediatric patients, maintaining visual function is crucial for normal development and learning, as well as for maintaining quality of life and independence.

Broad Implications for Retinal Diseases: The successful application of AAV5-CNGB1 could set a precedent for the treatment of other genetic retinal disorders. Demonstrating efficacy in this condition may encourage the development of similar gene therapies for a range of other retinal dystrophies, potentially transforming the treatment landscape for these challenging diseases.

Link between CNGB1 mutation and disease phenotype: *CNGB1* encodes three rod photoreceptor-specific transcripts; full length *CNGB1* (often known as *CNGB1a* to distinguish it from an alternative transcript expressed in olfactory epithelium; CNGB1b) and two shorter glutamic acid rich transcripts (GARP1 and GARP2). CNGB1 combines with CNGA1 (Cyclic Nucleotide-Gated Channel, Alpha 1), with a stoichiometry of 1:3, to form the CNG channel within the rod outer segment plasma membrane.[16,17] Assembly of the subunits occurs in the rod inner segment prior to trafficking of the CNG channel to the outer segment. The rod CNG channel is an essential component of the phototransduction cascade that mediates the translation of light-triggered changes in the second messenger cyclic guanosine monophosphate (cGMP) into a voltage and calcium signal. Binding of cGMP to the CNG channels leads to opening of a proportion of the channels and depolarization of the rod, and this is the situation in the dark- adapted state. Following light stimulation and hydrolysis of cGMP by rod phosphodiesterase (PDE6) activity, the CNG channels close, and the rod becomes hyperpolarized. This reduces the release of the neurotransmitter, glutamate, at the synaptic terminal, thus eliciting transretinal signaling. The CNGB1 protein has an N-terminal proline and glutamic acid rich region that interacts with peripherin/RDS. This interaction is involved in the correct outer membrane localization of the CNG channel.[18] Use of alternative exons results in translation of this N-terminal part of CNGB1 as separate short non-membrane bound GARP proteins. GARP1 is expressed at low levels and its functional role is not well characterized. The GARP2 protein plays a structural role in the rod outer segment, interacting with peripherin/RDS[19,20] and the PDE6 complex.[21] GARP2 is necessary for normal rod outer segment disc formation and maintenance. Through interactions with PDE6, GARP2 also contributes to the regulation of gain, and response recovery of the phototransduction cascade.[22]

In the retina, CNGB1 expression is restricted to rods; thus, the construct has been designed to selectively target this cell population. Preventing rod death is essential to preserving vision, as CNGB1-RP leads to secondary cone loss once rod cells are completely degenerated. Preventing rod degeneration prevents cone loss, preserving as much functional vision as possible.

4.2.1 Clinical Efficacy of AAV5-CNGB1

To date, no clinical data have been collected with AAV5-CNGB1 for the treatment of patients with Autosomal Recessive Retinitis Pigmentosa (arRP) due to CNGB1 mutations. However, preclinical studies in mice and dog models have shown promising results, with AAV5-mediated gene replacement therapy rescuing the structure and function of the retina in CNGB1 retinopathy.

A natural history study (NHS) is ongoing to longitudinally evaluate CNGB1 retinopathy biomarkers and their association with outcomes related to visual function, disease progression, and quality of life. This NHS, funded under grant R24EY027285, is being conducted at multiple sites, including Columbia University, LMU Munich, and Eberhard Karls University of Tuebingen. The study has enrolled a genetically diverse cohort of 26 participants aged 18 to 75 years, including a mix of early-stage and advanced disease patients.

The NHS aims to identify candidate biomarkers and surrogate endpoints for future clinical trials, providing a clinical benchmark for gene replacement therapy. Preliminary data from this study have highlighted key biomarkers such as ellipsoid zone (EZ) length and full-field stimulus testing (FST) responses, which are being used to inform the design and evaluation of the upcoming Phase I/IIa clinical trial for AAV5-CNGB1.

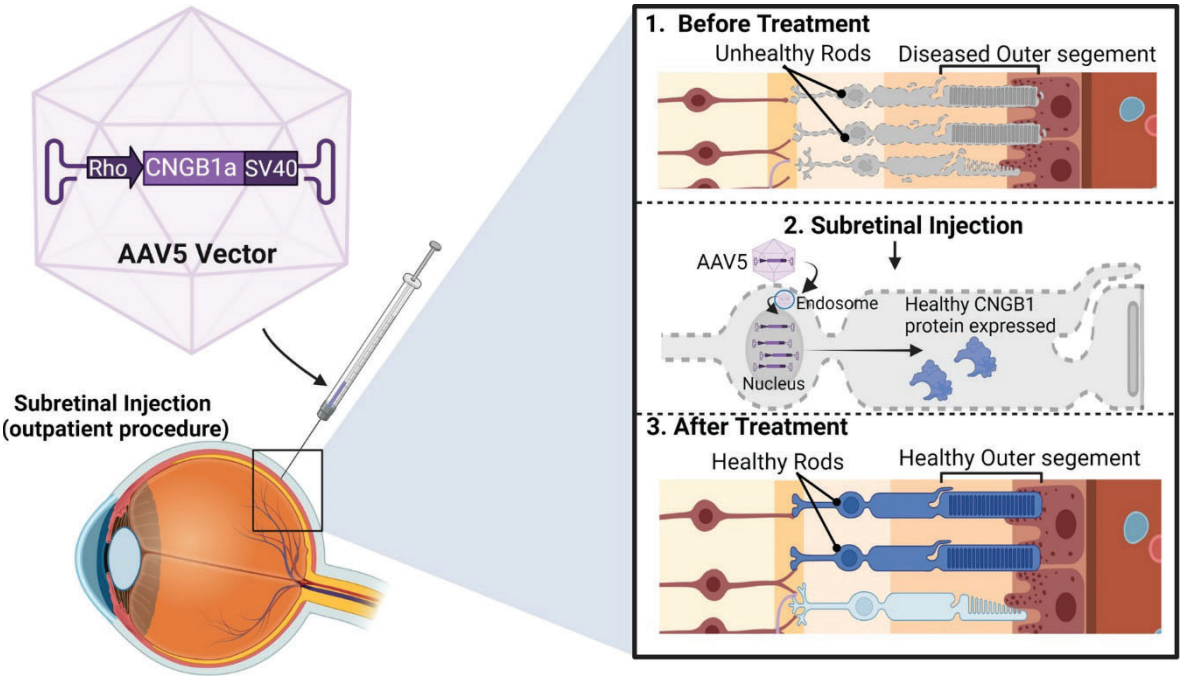
4.2.2 Nonclinical Efficacy of AAV5-CNGB1

Previous gene therapy in *Cngb1*$^{-/-}$ mouse and dog models resulted in sustained restoration of rod function and retinal structural preservation.[12,23,24] Untreated animals lack rod function and then progressively lose the non-functioning rods and secondarily the genetically normal cones. Subretinal delivery of species matched vectors in both animal models resulted in normal CNG channel formation in the rod outer segment within the treated area (approx. 1/5 to 1/3 of the total retinal area). This rescued rod function was determined by Ganzfeld electroretinography (ERG) and visual behavior testing. In addition, the therapy preserved photoreceptors and photoreceptor layer thickness and halted secondary degeneration of cone photoreceptors. Importantly, the treatment not only improved visual function and preserved retinal structure, but also improved functional vision as measured by vision-guided behavior.

Therapeutic Expression Construct: The AAV5-CNGB1 vector protein expression has been confirmed *in vivo* in the *Cngb1*$^{-/-}$ mouse and the *Cngb1*-deficient dog model.[12] Safety and biodistribution testing in non-human primates is complete and pending publication (**Fig. 5**)

Rationale for ubiquitous vs. tissue-specific promoter: The AAV5-*CNGB1* vector contains a short human rhodopsin (*RHO*) promoter, which has been shown to drive highly efficient, rod-specific gene expression in mice, dogs, and non-human primates. Thus, we can expect that AAV5-*CNGB1* will mimic the native cellular expression pattern of *CNGB1* in the human retina (**Fig. 3**). Ubiquitous protomers have the potential to drive expression of CNGB1 in non-target cells with unknown safety risks.

Fig. 3. Overview of AAV5-CNGB1 phase 1/2 trial. Patients will receive a subretinal injection of AAV5-CNGB1. The AAV5 vector enters rod cells, and the expression construct is trafficked to the nucleus whereafter it is used to make wild-type (healthy) CNGB1 protein, preserving rod survival and slowing cone visual decline.



Rationale for single-stranded versus self-complementary design: The only option is to use a single stranded design because CNGB1 cDNA is too large to be packaged efficiently in a self-complementary vector. Furthermore, the single strand design is demonstrated to have successful and effective delivery in preclinical models.[12]

Codon-optimization status of the expression construct: The CNGB1 codon sequence has not been codon optimized to best mimic the endogenous transcript. Effects of codon optimization cannot always be predicted. Since the non-codon optimized sequence of CNGB1 is stable and supports high level gene expression, we opted not to perform codon optimization. Also, given that CNGB1 localizes in rods only, we will not be able to non-invasively measure expression in patients and thus have no need to use codon optimization to facilitate detection in assays.

Availability of antibodies to distinguish the human transgene? Antibodies that detect human CNGB1 protein are available. Due to the high level of homology of CNGB1 protein across species and the challenge to generate good antibodies against transmembrane proteins, such as CNGB1, it has so far not been possible to generate antibodies of high quality that are suitable for discrimination across species.

Nonclinical species selection rational: Small animal (mouse) and large animal (dog) models of CNGB1-RP both recapitulate the clinical phenotype of the human disease[12]. These models are well suited for pharmacodynamics studies. Safety studies should optimally mimic the human ocular specifics (size, structure and immunology). Therefore, a large animal model with eye size and structure most similar to the human eye would be

preferred. Examples are the dog model (with a naturally occurring CNGB1 mutation) or the non-human primate model (a primate with CNGB1 mutation is not available). The model could be chosen based on availability.

[redacted]

Nonclinical studies conducted to date:

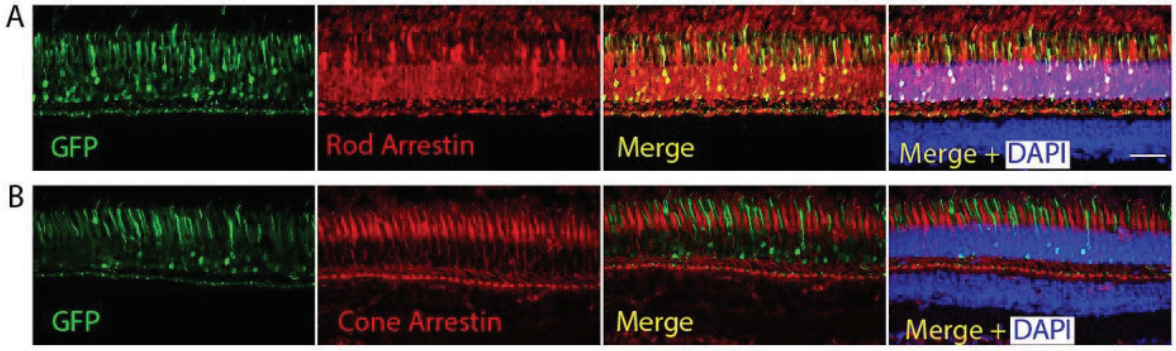
Our team Profs Michalakis and Petersen-Jones have conducted proof-of-concept studies in an engineered mouse and spontaneously occurring dog model of *CNGB1*-RP.[12,18,24,29]

Mouse: We have recently reported on rescue of rod function in the *Cngb1*-X26 mouse model using an AAV serotype 5 construct with human *CNGB1* cDNA driven by a novel short human *RHO* promoter.[30]

Canine: Using the same construct proposed in this Phase I/IIa clinical trial, we demonstrated efficacy in a pivotal, preclinical study in a canine model (manuscript in preparation). The study met its pre-specified primary endpoint, as subjects in the experimental group showed significant rescue compared to controls after 1 year. Improvements in the experimental group were apparent as early as 3 months after treatment and were sustained over the 18 months of observation, while controls showed no improvement during this time (**Fig. 4**). Subjects in the Control/Intervention group, once transduced with AAV5-*CNGB1*, demonstrated a comparable onset and durability in

visual performance improvements to those observed in the Original Intervention group, through at least 18 months following sequential therapy administration.

Fig. 5. AAV5-CNGB1 injected in non-human primates specifically localizes to the rods and is not detected in any non-ocular tissue. (A) Rod Arrestin (red fluorescence) colocalization with AAV5-CNGB1 expression (labeled with green fluorescence protein). (B) Cone Arrestin (red fluorescence) staining does not overlap with AAV5- CNGB1 expression (labeled with green fluorescence protein).



5 ORPHAN DRUG STATUS

Current Status and Justification: AAV5-CNGB1 is proposed for designation as an orphan drug for the treatment of autosomal recessive retinitis pigmentosa due to CNGB1 mutations, a condition affecting a small patient population with serious and life-altering implications. As per FDA guidelines, an orphan drug is defined as a treatment for a disease or condition that affects fewer than 200,000 people in the United States. Given the rarity of CNGB1-related retinitis pigmentosa and the specificity of this genetic target, AAV5-CNGB1 meets the criteria for orphan drug status.

Lack of Existing Treatments: Currently, there are no FDA-approved treatments that specifically address the underlying genetic defect in patients with CNGB1 mutations. The available interventions for retinitis pigmentosa, such as vitamin A supplementation, are supportive at best and do not halt disease progression nor restore vision. AAV5-CNGB1's development is particularly significant as it offers a targeted genetic correction, potentially halting or significantly slowing the progression of photoreceptor degeneration directly related to CNGB1 mutations [16].

Potential Clinical Superiority: In addition to its eligibility for orphan drug status due to the rarity of the targeted condition, AAV5-CNGB1 may also be considered clinically superior to any existing therapies for retinitis pigmentosa that may receive orphan designation in the future. This is because AAV5-CNGB1 directly addresses the genetic root of the disease rather than merely alleviating symptoms or slowing down the degenerative process without altering its course [16].

Regulatory and Developmental Implications: Obtaining orphan drug designation for AAV5-CNGB1 would support the therapy's development through several regulatory advantages, including tax credits for clinical testing, exemption from FDA application fees, and potential

eligibility for seven years of market exclusivity upon approval. These benefits are critical for encouraging the development and ensuring the availability of a treatment option for a condition with such a profound impact on children's lives.

Commitment to Addressing Unmet Needs: The pursuit of orphan drug status for AAV5-CNGB1 underscores our commitment to addressing the unmet medical needs within a vulnerable population. By focusing on a rare genetic cause of blindness, this therapy not only promises to enhance the quality of life for affected individuals but also contributes to the broader field of genetic and personalized medicine.

6 PATIENT SUBSET CONSIDERATIONS AND MEDICAL PLAUSIBILITY OF THE CHOSEN SUBSET

Identification of the Patient Subset: The targeted patient subset for AAV5-CNGB1 gene therapy comprises individuals with autosomal recessive retinitis pigmentosa specifically due to biallelic mutations in the CNGB1 gene. This genetic specificity is critical as the therapy's mechanism of action—gene replacement—is directly designed to compensate for the dysfunctional or absent CNGB1 protein, which is pivotal in the phototransduction process of rod photoreceptors in the retina.

Medical Plausibility for Targeting this Subset: The choice to focus on this particular subset is medically plausible for several reasons:

1. Genetic Specificity: The therapy uses a viral vector to deliver a functional copy of the CNGB1 gene, directly addressing the root cause of the disease in these patients. By targeting the genetic deficit precisely, the treatment can potentially restore normal function at the cellular level, leading to clinically meaningful improvements in visual function or a deceleration of disease progression.

2. Unmet Medical Need: Patients with CNGB1-related retinitis pigmentosa represent a group with significant unmet medical needs. Currently, there are no effective treatments available that can halt or reverse the vision loss associated with their condition. Providing a therapy that could offer visual stabilization or improvement would have a profound impact on their quality of life and ability to function independently.

3. Potential for Efficacy: Based on preclinical studies, the chosen patient subset is likely to respond to gene therapy due to the still present, albeit non-functional, photoreceptors [2,15-19]. Early intervention could preserve these cells before the disease progresses to the point of irreversible cellular death, enhancing the likelihood of successful treatment outcomes.

4. Feasibility of Clinical Evaluation: By clearly defining the patient subset, clinical trials can be more effectively designed with stringent inclusion criteria, ensuring that the therapy's effects are evaluated in those most likely to benefit. This targeted approach enhances the robustness and reliability of clinical data, facilitating regulatory review and potential approval processes.

Justification for Exclusivity in the Subset: AAV5-CNGB1 gene therapy is designed specifically for CNGB1 mutation carriers, meaning it would not be applicable or beneficial for patients with retinitis pigmentosa caused by other genetic mutations. This exclusivity is justified because the therapeutic gene carried by the AAV5 vector will only correct defects directly associated with CNGB1 abnormalities. Other forms of retinitis pigmentosa involve different pathophysiological mechanisms that would not be addressed by this therapy.

7 REGULATORY STATUS AND MARKETING HISTORY

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 studies and review the completed proof of concept preclinical studies. We are targeting IND application submission for fall of 2025.

AAV5-CNGB1 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 AAV5-CNGB1 for the same rare disease or condition prior to submission of the AAV5-CNGB1 orphan drug designation request.

8 DOCUMENTATION OF PATIENT POPULATION SIZE

Autosomal recessive retinitis pigmentosa due to CNGB1 mutations is estimated to affect fewer than 200,000 people in the United States, qualifying it as a rare disease under FDA guidelines.

Patient Population Size

The determination of the patient population size for autosomal recessive retinitis pigmentosa due to CNGB1 mutations is critical for understanding the scope of impact of the proposed gene therapy and justifying its designation as a treatment for a rare disease. According to FDA guidelines, a rare disease affects fewer than 200,000 individuals in the United States.

Estimation Methodology: The prevalence of autosomal recessive retinitis pigmentosa due to CNGB1 mutations is estimated by reviewing published genetic studies and patient registry data that detail the incidence of this specific mutation among those diagnosed with retinitis pigmentosa. Retinitis pigmentosa itself affects approximately 1 in 4,000 individuals in the United States, translating to about 82,500 people. CNGB1 mutations are responsible for approximately 2-4% of all retinitis pigmentosa cases, according to genetic screening studies [5-7].

Data Sources: The patient population size has been estimated using several key sources:

Genetic and Epidemiological Research: Peer-reviewed articles and studies that analyze the genetic landscape of retinitis pigmentosa provide insights into the frequency of CNGB1 mutations [8-11].

Patient Registries: Registries that track retinitis pigmentosa patients, such as those maintained by major ophthalmology research centers and patient advocacy organizations, offer data on the prevalence of specific genetic mutations, including CNGB1.

Clinical Data: Data from ongoing clinical trials and genetic testing services also contribute to understanding the distribution of CNGB1 mutations within the broader population of those affected by retinitis pigmentosa.

Estimated Population Size: From these data, it is estimated that between 1,650 and 3,300 individuals in the United States suffer from retinitis pigmentosa due to CNGB1 mutations. This estimate places autosomal recessive retinitis pigmentosa due to CNGB1 mutations well within the criteria for a rare disease as defined by the FDA, affecting significantly fewer than 200,000 people nationwide.

Summary: The patient population size for autosomal recessive retinitis pigmentosa due to CNGB1 mutations is demonstrably rare, underscoring the need for targeted therapeutic interventions such as AAV5-CNGB1. The rarity and specificity of this genetic mutation necessitate the development of specialized treatments to address the unmet medical needs of this patient group.

9 RARE PEDIATRIC DISEASE DESIGNATION JUSTIFICATION

Given the profoundly debilitating nature of autosomal recessive retinitis pigmentosa (RP) due to CNGB1 mutations, and the complete lack of effective treatments, we advocate for the designation of AAV5-CNGB1 as a Rare Pediatric Drug Product. This gene therapy represents a groundbreaking approach with the potential to significantly alter the course of the disease by providing therapeutic benefits early in life, when they can have the most profound impact.

Serious and Debilitating Nature of the Disease:

Autosomal recessive retinitis pigmentosa triggered by CNGB1 mutations typically manifests in early childhood, leading to a rapid progression from night blindness to complete vision loss during critical developmental years. This progression not only hinders physical and educational development but also imposes severe emotional and economic strains on families. The transition to blindness during childhood can severely limit future opportunities in academic and social settings, impacting the child's overall potential and quality of life.

Lack of Effective Treatments:

Currently, there are no FDA-approved therapies that address the genetic basis of CNGB1 mutations, with existing treatments only offering symptomatic relief. AAV5-CNGB1 introduces a functional copy of the CNGB1 gene directly to the affected retinal cells, presenting a unique approach that could potentially restore vision. This method directly targets the underlying cause of the disease, making it a critical development in the field of genetic therapy for pediatric conditions.

Transformative Potential of AAV5-CNGB1:

Evidence from preclinical studies suggests that AAV5-CNGB1 can significantly preserve and potentially improve retinal function, offering hope for not just halting but reversing the effects of degeneration. The ability of this therapy to intervene effectively during early childhood is vital, as it could dramatically change the disease's trajectory, allowing children to retain and possibly regain vision.

Emphasis on Pediatric Impact:

Designating AAV5-CNGB1 as a Rare Pediatric Disease Product is particularly crucial because autosomal recessive retinitis pigmentosa (ARRP) due to CNGB1 mutations manifests and progresses during childhood—a period when vision is essential for learning, social interaction, and

overall development. The disease typically presents in early childhood with initial symptoms such as night blindness and loss of peripheral vision, usually detectable between the ages of 5 and 10. As the disease progresses, children experience a gradual constriction of their visual field, commonly referred to as tunnel vision, which significantly impacts their daily activities and quality of life.

By adolescence, many affected individuals have severe visual impairment, making it difficult to perform tasks that require fine visual acuity. The progression from early symptoms to severe visual impairment is typically relentless, with most individuals experiencing profound vision loss by their late teens to early twenties. This rapid progression during crucial developmental years profoundly affects a child's ability to participate in educational activities, social interactions, and physical activities, leading to significant developmental delays and psychological challenges.

The therapeutic benefits of early intervention with AAV5-CNGB1 could provide children with the opportunity to maintain their vision, thus preserving their ability to engage fully in educational and social activities. Early intervention with AAV5-CNGB1 aims to halt or significantly slow the degeneration of photoreceptors, preserving both rod and cone function. This preservation is critical during childhood and adolescence, as maintaining visual function during these years can support normal learning and development, reduce the need for special educational services, and improve overall quality of life.

Early intervention is not just about treating a disease but about giving children a chance at a normal and fulfilling childhood. By preserving vision, children can engage in typical activities such as reading, playing sports, and participating in social events. This can significantly improve their self-esteem, social skills, and academic performance, leading to better lifelong prospects. The ability to maintain vision can also reduce the emotional and financial burden on families, as they would not have to navigate the challenges associated with raising a visually impaired child.

In summary, the early onset and rapid progression of vision loss due to CNGB1 mutations justify the Rare Pediatric Disease Designation for AAV5-CNGB1. This therapy offers a novel and direct method for correcting a genetic defect, with the potential to transform the lives of affected children by preserving their vision during critical developmental years. Granting this designation emphasizes the urgent need for innovative, effective treatments that address the genetic underpinnings of debilitating pediatric conditions, reflecting our commitment to enhancing the lives of affected children and their families. Through the development of AAV5-CNGB1, we aim to transform the landscape of treatment for autosomal recessive retinitis pigmentosa and similar pediatric diseases.

Urgency for Expedited Development:

The designation of AAV5-CNGB1 as a Rare Pediatric Disease Product is essential for facilitating its rapid development and review. This status would provide access to incentives, such as the Priority Review Voucher, accelerating the therapy’s path through regulatory processes and bringing it closer to clinical availability. Early and effective treatment is crucial for these young patients, as it can prevent irreversible damage and vastly improve developmental outcomes.

Summary:

The early onset and rapid progression of vision loss due to CNGB1 mutations justify the Rare Pediatric Disease Designation for AAV5-CNGB1. This therapy not only offers a novel and direct method for correcting a genetic defect but also sets a new standard for treating severe pediatric

genetic disorders. Granting this designation emphasizes the critical need for innovative, effective treatments that address the genetic underpinnings of debilitating pediatric conditions, reflecting our commitment to enhancing the lives of affected children and their families. Through the development of AAV5-CNGB1, we aim to transform the landscape of treatment for autosomal recessive retinitis pigmentosa and similar pediatric diseases.

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11 APPENDIX A: NUCLEOTIDE TRANSGENE SEQUENCE OF *CNGB1*

ATGTTGGGCTGGGTCCAGAGGGTGCTGCCTCAGCCCCCAGGGACCCCTCGGAAGAC
CAAGATGCAGGAGGAAGAGGAAGTGGAACCAGAGCCAGAGATGGAGGCGGAGGTG
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AGAGTCATTCAAGGAGGAGGAAGTGGCTGTGGCAGACCCAAGCCCTCAGGAGACCA
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TGGCAGCACTGGGGACACAGGGTGCACAGATGAACCCAATGAGGCCCTTGAGGCCC
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