Molecular Therapy
Review

Recombinant adeno-associated virus as a delivery platform for ocular gene therapy: A comprehensive review

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Adeno-associated virus (AAV) has emerged as a leading platform for *in vivo* gene therapy, particularly in ocular diseases. AAV-based therapies are characterized by low pathogenicity and broad tissue tropism and have demonstrated clinical success, as exemplified by voretigene neparvovec-rzyl (Luxturna) being the first gene therapy to be approved by the U.S. Food and Drug Administration to treat *RPE65*-associated Leber congenital amaurosis (LCA). However, several challenges remain in the development of AAV-based gene therapies, including immune responses, limited cargo capacity, and the need for enhanced transduction efficiency, especially for intravitreal delivery to photoreceptors and retinal pigment epithelium cells. This review explores the biology of AAVs in the context of gene therapy, innovations in capsid engineering, and clinical advancements in AAV-based ocular gene therapy. We highlight ongoing clinical trials targeting inherited retinal diseases and acquired conditions, discuss immune-related limitations, and examine novel strategies for enhancing AAV vector performance to address current barriers.

INTRODUCTION

Adeno-associated virus (AAV) was first discovered as a contaminant in an adenovirus preparation by Bob Atchison,[1] M. David Hogganm and Wallace P. Rowe in the mid-1960s.[2] Further studies revealed that AAV contains a single-stranded DNA (ssDNA) genome[3] flanked by self-priming hairpins, which are termed inverted terminal repeats (ITRs).[4–6] The wild-type (wt) AAV genome may integrate into the host chromosome or exist extra-chromosomally as episomes, and can express transgenes in either state.[7–9] With oversight from the National Institutes of Health Recombinant DNA Advisory Committee, cloning the AAV genome into plasmids was achieved, which was an important milestone for the field.[10–12] These pioneering works laid the foundation for using recombinant AAV (rAAV) as a gene therapy delivery platform.

Gene therapy has emerged as a treatment modality for human diseases that involves the delivery and/or modification of genetic material inside target cells. A main approach is gene replacement therapy, which introduces a functional copy of a defective gene into cells to restore its function.[13,14] Conversely, gene silencing therapy, also known as RNA interference (RNAi) therapy, is a standard approach to treat genes that have toxic gain-of-function mutations by utilizing small RNA molecules to suppress or silence the defective gene.[13,14] Gene editing therapy has become a feasible gene therapy approach with the innovation of CRISPR-Cas9 technology.[15] This was further advanced by the invention of prime and base editing,[16,17] which involve direct modification of DNA without inducing double-strand breaks in the DNA. Modified CRISPR systems have also been developed to allow transient RNA editing.[18] In addition, gene expression can be epigenetically altered by DNA methylation,[19] histone modification,[20] and microRNA regulation.[21] Other emerging strategies for addressing specific types of mutations are being developed, such as suppressor tRNAs facilitating readthrough of premature stop codons caused by nonsense mutations.[22,23] Regardless of the strategy being used, gene therapy can be implemented either *ex vivo* or *in vivo*. *Ex vivo* gene therapy involves isolating the target cells from a patient, genetically modifying them outside the body, and then reintroducing them to the patient while *in vivo* gene therapy delivers genetic materials directly to the target tissues. rAAVs represent the leading *in vivo* delivery platform for many treatment modalities owing to low pathogenicity, broad tissue tropism, and relatively high delivery efficacy, and are widely used in both laboratories and clinical trials.

The eye has many characteristics that make it a favorable target for rAAV-based gene therapy, such as its compartmentalization from the rest of the body for vector containment, small volume necessitating less vector use, easy accessibility for vector delivery, and immune privileged status, protecting vectors from host immune responses.[24–28] Additionally, the eye can be easily imaged—techniques such as

https://doi.org/10.1016/j.ymthe.2024.10.017.

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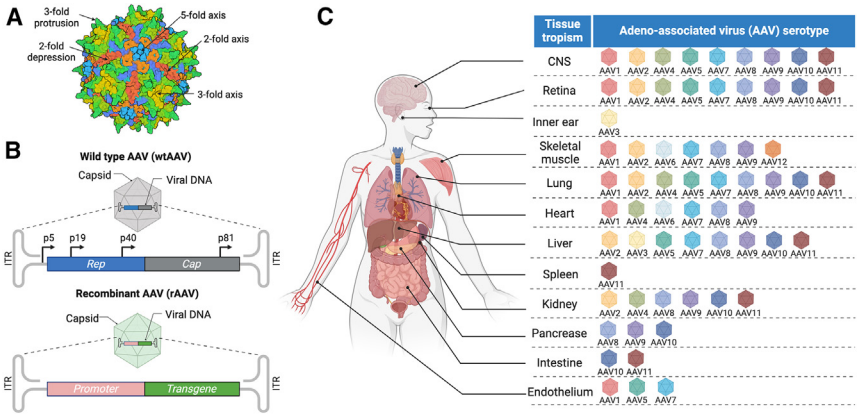

Figure 1. Characterization of AAV capsid structures, genomes, and tissue tropisms

(A) AAV capsid surface model demonstrates the icosahedral 2-, 3- and 5-fold axes. (B) Genome structure of wtAAV and rAAV. Within the wtAAV genome, the transcription of *Rep78* and *Rep52* genes is regulated by promoters *p5* and *p19*, respectively. These transcripts can undergo alternative splicing, producing two shorter transcripts, *Rep68* and *Rep40*. Additionally, the transcription of the *Cap* gene is regulated by promoter *p40*. The Cap transcripts undergo alternative splicing, resulting in three viral structural proteins: VP1, VP2, and VP3. The rAAV genome is created by replacing viral genes with an expression cassette containing the transgene of interest flanked by two ITRs, the sole *cis* element necessary for DNA replication and packaging. rAAV production is accomplished by providing *Rep*, *Cap* and, *Ad* helper gene functions *in trans*. (C) Preferential tissue tropisms of natural AAV serotypes.

electroretinography allow for the measurement of functional outcomes to evaluate gene therapy efficacies—and comparisons between treatment and control conditions can be done between eyes in the same animal. These advantages led to the U.S. Food and Drug Administration (FDA) approving voretigene neparvovec-rzyl (Luxturna) as the first rAAV-based gene therapy for ocular use, providing a novel treatment for patients with biallelic *RPE65* mutations.[29] Since then, several rAAV-based gene therapy products have received regulatory approval for treating various diseases, such as spinal muscular atrophy,[30] aromatic L-amino acid decarboxylase deficiency,[31] Duchenne muscular dystrophy,[32] hemophilia A,[33] and hemophilia B.[34] Despite its clinical success, rAAV still faces several limitations and challenges as a delivery platform for ocular gene therapy, including vector-induced inflammation, transduction efficacy, tissue/cell type specificity, cargo capacity, and long-term efficacy.

In this review, we delve into the biology of AAV in the context of gene therapy, and its application in ocular gene therapy. As of this writing, this review also includes updates from recent developments in capsid engineering and perspectives on the most recent results from clinical trials.

AAV BIOLOGY AND MECHANISMS

Genetics and structure of AAV

AAV is a small, non-enveloped virus with an icosahedral 60-mer capsid (Figure 1A). Its genome consists of a 4.7-kb ssDNA molecule.[35] The wtAAV genome contains *cap* and *rep* genes flanked by ITRs, which are essential for replication and packaging during the viral life cycle.[36] In rAAV gene therapy, these viral genes are removed to make vector replication incompetent and maximize the amount of DNA that can be packaged into the vector for gene replacement, silencing, editing, and/or regulation.

The capsid determines the tropism of AAV vectors as the amino acid residues on the surface of the capsid interact with receptors on host cells. The binding between capsid proteins and specific receptors on the target cells dictate the virus's ability to enter and transduce cells.[13,14,37,38] The capsid is also the primary target for the neutralizing antibodies (NAbs), which are produced by the adaptive immune response and can bind to the virus and block its entry into cells. These NAbs can be generated in response to the vector being introduced into the host or could already be present in the body from previous exposure to naturally occurring AAVs. The eye is an immune-privileged environment that is protected from circulating NAbs, which allows AAV vectors to persist in the host for the persistent expression of the cargo transgenes as a non-integrating episome.[9] However, in some cases AAV-delivered genes can integrate into the host genome, which is typically at the *AAVS1* site on chromosome 19; however, this occurs at a very low frequency.[39]

Therapeutic transgenes and regulatory elements, such as the promoter and polyadenylation signal, must fit within the approximately 4.5-kb capacity of the rAAV genome between the necessary ITRs (Figure 1B). Different AAV serotypes, which have been isolated from adenovirus stocks or primate tissues, have distinct tropisms (Figure 1C).[13,40,41] For example, AAV2 and AAV8 are frequently used for retinal gene delivery due to their ability to transduce retinal cells. However, the structure-function relationships that determine tropism are not completely understood. Beyond the physical properties of the capsid, factors such as expression and modification of cell surface receptors, intracellular trafficking, nuclear entry, and rate of second-strand DNA synthesis also influence AAV transduction efficiency.

While AAV infections are generally asymptomatic,[42–45] recent reports have linked AAV2 to pediatric hepatitis outbreaks during the COVID pandemic.[46,47] Despite this, AAV remains a versatile tool for gene therapy, with its genomic diversity driven by variable regions in the *cap* gene, enabling targeted delivery across different tissues.

Mechanism of AAV-mediated gene delivery

AAV-mediated gene delivery involves a sophisticated sequence of events that begins with the viral capsid binding to specific receptors on the target cell surface, a process that is largely determined by the

capsid's structure. Common receptors utilized by different AAV serotypes include the heparan sulfate proteoglycan (HSPG) for AAV2; sialic acid for AAV1, AAV4, AAV5, and AAV6; and N-linked galactose for AAV9. Co-receptors, such as integrins and fibroblast growth factor receptors, may also be involved in facilitating the virus's attachment and internalization into the cell.[13,48–50] Receptor-binding sites on the AAV capsid play a critical role in determining the virus's cell and tissue tropism, making them a focal point for engineering efforts aimed at enhancing transduction efficiency in specific target cells. Recent genome-wide screening studies have revealed that many host proteins in addition to glycans are essential for rAAV cellular entry. Notably, the type I transmembrane protein KIAA0319L was identified as the AAV receptor (AAVR) and is universally required for the transduction of most rAAVs.[51] Additionally, GPR108 has been recognized as a crucial cell entry factor for several rAAV serotypes.[52] Knockout studies of either AAVR or GPR108 in cells and mice have shown that these proteins do not significantly affect rAAV binding to the cell surface,[52,53] but they are vital for successful transduction, particularly in the post-attachment steps of the viral entry process.

rAAV capsids attach to cells and are then internalized by endocytosis via clathrin- and caveolin-dependent as well as independent mechanisms into endosomes within the cell.[48] As the endosome matures and its environment acidifies, the rAAV capsid undergoes conformational changes that allow it to escape into the cytoplasm and avoid degradation.[54,55] The small size of AAV enables it to pass through the nuclear pore complex, where it enters the nucleus intact before releasing its ssDNA genome.[56,57] For gene expression to occur, the single-stranded viral DNA must be converted into a double-stranded form by host cell enzymes, which is often the rate-limiting step in transduction.[58] Some rAAVs have been designed with self-complementary genomes to accelerate this step, but this reduces the packaging capacity by one-half.[59,60] The therapeutic genes are then expressed from the double-stranded DNA template.

While rAAVs generally elicit a low innate immune response and little cytokine expression is observed immediately after administration, the adaptive response against rAAVs can be potent, leading to the production of NAbs against the viral capsid or the transgene product.[61,62] This immune response can limit the effectiveness of the therapy, making it essential to carefully select serotypes and design vectors that minimize immunogenicity.

rAAV AS A VECTOR FOR OCULAR GENE THERAPY

Advantages of rAAV in ocular applications

rAAVs are exceptionally well-suited for ocular gene therapy. The immune-privileged environment of the eye[63] combined with the low immunogenicity of rAAV not eliciting strong innate immune responses minimizes the risk of adverse immune reactions. Moreover, rAAVs can target a wide range of retinal cells, including photoreceptors, retinal pigment epithelium (RPE), and ganglion cells, and various engineered AAV capsids have been developed to optimize transduction of specific cell types. The small size of rAAV facilitates effective diffusion through the retinal layers, making it suitable for both subretinal and intravitreal injections. Additionally, the small volume of the eye significantly reduces both manufacturing and therapeutic costs for clinical-grade rAAV production compared strategies that target larger compartments such as the brain or require systemic administration.

Serotype selection for ocular gene therapy

AAV2 is one of the most extensively studied and utilized serotypes in ocular gene therapy. Its strong affinity for HSPG on retinal cells makes it particularly effective for nearly all retinal cell types, but different cell types can be targeted, depending on the administration route. Thus, AAV2 has been the vector used in many successful clinical trials, including those targeting Leber congenital amaurosis (LCA) and choroideremia. Other commonly used serotypes include AAV5 and AAV8, which each offer distinct advantages. AAV5 is less seroprevalent among human populations and is, therefore, less likely to be blocked by pre-existing NAbs.[64] A phase 1/2 study found that subretinal delivery of AAV5-hRKp.RPGR was safe, well tolerated, and improved retinal sensitivity and functional vision in males with XLRP-RPGR, supporting further phase 3 trials.[65] AAV8 has been found to be better able to efficiently transduce photoreceptors than AAV2 when administered by subretinal injection.[66]

Recent advancements in capsid engineering have led to the development of novel AAV serotypes with enhanced transduction efficiency, reduced immunogenicity, and greater specificity for targeted retinal cells. Engineered variants such as AAV2.7m8 and Anc80 have shown promising results in preclinical studies, each demonstrating superior transduction of different retinal cells compared to their natural counterparts.[67–69] These variants are typically developed through directed evolution or rational design, allowing for the fine tuning of capsid properties to meet specific therapeutic needs. AAV2.7m8 has received regulatory approval in clinical trials for treating age-related macular degeneration (AMD) and diabetic macular edema (DME),[70] establishing that novel engineered capsids can be used to target gene therapy to specific retinal cells through intravitreal injections, which are less invasive and more convenient than subretinal injections.

Promoters and regulatory elements

A critical aspect of rAAV design is the selection of promoters and regulatory elements that drive transgene expression at appropriate levels in specific cell types within the eye. Cell-specific promoters, such as the rhodopsin promoter for rod photoreceptors and the G protein-coupled receptor kinase 1 (GRK1) promoter for both rod and cone photoreceptors, minimize off-target effects and maximize therapeutic efficacy and promoters like *RPE65* or bestrophin 1 (*BEST1*) can drive expression in RPE cells. For applications requiring broad and robust gene expression, strong ubiquitous promoters like the cytomegalovirus (CMV), chicken beta-actin (CBA), or hybrid CBA promoters (CAG) are used.

Other regulatory elements in addition to the promoter can enhance transgene expression. The woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) is commonly included in gene

therapy vectors to stabilize mRNA and boost translation. However, this may have unintended consequences, such as increased oncogenic potential in lentiviral vectors, which can be mitigated by removing the risky sequences from the WPRE.[71,72] rAAV vectors typically contain a Kozak sequence as a translational enhancer to enhance efficient translation initiation and a specific polyadenylation signal, such as the bovine growth hormone polyA tail, for mRNA stability and export to improve sustained expression.[13] Furthermore, incorporating miRNA-mediated regulation can further control gene expression by incorporating miRNA target sites into the transgene's 3′ UTR, which will silence the transgene in tissues expressing the complementary miRNA to enable more precise, cell-specific expression. For example, an rAAV5 vector incorporating miR-124 and miR-204 target sequences effectively restricted transgene expression to RPE and photoreceptor cells in mice and pigs, even at low doses.[73]

Capsid engineering for intravitreal injection

Currently, rAAV-based ocular gene therapy predominantly uses subretinal injection, through which rAAV can reach photoreceptor and RPE cells, the outer retinal cell types which are both associated with numerous inherited retinal diseases (IRDs).[74] While subretinal injection has the advantage of being comparatively easy to access the intended cells, this route of administration requires highly skilled surgeons, significant time and costs, complicated logistical challenges, and a risk that the surgical bleb created during injection can cause retinal detachment, especially in patients with already degenerating retinas. Due to the confined space of the bleb formed during delivery, rAAV only infects a restricted number of photoreceptors and RPE cells, resulting in limited transduction efficacy. Alternatively, intravitreal injection is a relatively easy and safe administration route to achieve broad dispersion of rAAV in the retina. Importantly, this procedure can be performed in a clinical office setting, reducing the cost and complexity of treatment. Despite these advantages over subretinal injection, a critical barrier significantly restricting its clinical application is that intravitreal injection results in transduction that is limited to the inner retina. This is thought to be caused by the strong binding between enriched HSPG in the inner limiting membrane (ILM) (the structural boundary between the vitreous and the retina) and the rAAV2 capsid limiting diffusion of the vector.[75] Thus, modifying the capsid to reduce this binding affinity could enhance the vector's ability to bypass the inner retina and diffuse into outer retinal layers to enhance its transduction in this part of the eye. Capsid engineering mainly consists of two approaches: rational design[76–78] and directed evolution.[67,79,80] However, other approaches such as isolating natural variants and utilizing machine learning (ML)-assisted capsid design have also been used to develop capsid variants capable of enhancing photoreceptor transduction via intravitreal injection.

Rational design

As our understanding of the structure and biology of rAAV has advanced, a sophisticated rational design approach has emerged to target specific sites within the AAV capsid genes. Epidermal growth factor receptor protein tyrosine kinases have been found to phosphorylate specific residues on the rAAV2 capsid surface, which triggers ubiquitination and degradation of rAAV2 by the proteasome before entering the nucleus to prevent it from expressing its transgenes.[76] To overcome this limitation, three surface-exposed tyrosine (Y) residues were replaced with phenylalanine (F) residues (Y444F/Y500F/Y730F) in VP3 of rAAV2. This modification led to enhanced transduction within the CNS.[81] Additional modifications of Y272F/Y444F/Y500F/Y730F/T491V (referred to as QuadYF+TV) resulted in a remarkable improvement of transduction in mouse retina. QuadYF+TV AAV2 vectors reach up to 25% of photoreceptors following intravitreal injection, whereas wtAAV2 was not able to transduce photoreceptors.[82] Despite this success, the mechanism of how these mutations contribute to increased transduction in outer retinal cells remains elusive. A recent study reported that the rationally designed capsid AAVv128 demonstrates significantly enhanced transduction efficiency and broader retinal tissue distribution compared to AAV8, with which it shares 99% sequence similarity, and specifically targets photoreceptors and RPE regardless of the administration route (subretinal, intravitreal, or suprachoroidal injection). Additionally, AAVv128 shows superior efficacy in inhibiting choroidal neovascularization in neovascular AMD and cryo-EM analysis that reveals unique structural features facilitating improved AAV binding, nuclear uptake, and endosome escape.[83]

Directed evolution

Directed evolution uses a relatively unbiased selective pressure mechanism on capsids to identify variants with favorable properties, such as increased rAAV yield, improved transduction, and/or specific cell/tissue tropism.[13,14] This process inserts random short-length peptides into rAAV capsids and can be done within a relatively short and controllable time frame. Engineering AAV2 capsids through modifying the HSPG binding sites, such as R585 and R588, has demonstrated that a certain extent of HSPG binding is required for rAAV2 diffusion, but strong HSPG binding traps the vector in the ILM and prevents its diffusion into outer retinal layers. One study generated an rAAV library using directed evolution to insert a random 7-mer peptide into the 3-fold protrusion of rAAV2 at N587 for intravitreal injection in mice.[67] Following multiple rounds of selection, an AAV2 variant termed 7m8 was identified that exhibited a robust capability for transducing both photoreceptors and RPE cells in mice as well as a limited number of photoreceptors in non-human primates (NHPs),[49] indicating its potential applicability in humans. Additionally, a chimeric rAAV2 incorporating modifications of 7m8 and QuadYF+TV exhibited markedly increased transduction in both inner retinal cells and photoreceptors in mice compared to an rAAV2 containing individual mutations.[77]

Similarly, another study adopted the same approach by pooling a variety of rAAV2-derived libraries and intravitreally injecting them into the eyes of NHPs.[79] The variant AAV2-LALIQDSMRA was identified after six rounds of selection that can be packaged at high titers (approximately 5.0×10^{13} viral genomes [vg]/mL) and was significantly enriched in the RPE. In comparison with the previously

described variant 7m8, which was selected in mice,[67] AAV2-LALIQDSMRA transduced fewer retinal ganglion cells but more photoreceptors (11.71-fold increase compared to 7m8) in NHPs, indicating that it may have stronger tropism in primate photoreceptors. In contrast to previous studies, Büning's group first counter-selected an AAV2-7-mer library for HSPG binding *in vitro* (i.e., selected for variants with reduced HSPG binding) before intravenous injection into mice in order to identify AAV2 variants enriched in the photoreceptors.[80] The selection process was repeated three times, leading to the identification of two variants, namely AAV2.GL and AAV2.NN. Both novel AAV2 variants showed strong and widespread retinal transduction after intravitreal injection in mice, dogs, and NHPs. In a combination of directed evolution and rational design approaches, Boye's group performed two rounds selection of AAV2-based library with simultaneous mutations across six surface-exposed variable regions in NHPs.[78] One capsid variant isolated from this screen, P2-V1, showed enhanced retinal cell transduction by intravitreal injection and capability of evading NAbs in human vitreous samples compared to AAV2 and 7m8. While these engineered vectors have overall been found to be safe and effective in small animal models, they are thought to require approximately 10- to 100-fold higher dose levels to achieve the same effects in large animals because of the larger eyeball volume to surface area ratio and distances that need to be crossed by diffusion.[67] This increases the potential to cause dose-dependent toxicity to the retina.

Directed evolution to screen for capsids with enhanced targeting of different tissues has primarily been conducted in mice and has led to the identification of several high-profile capsid variants with significantly enhanced transduction capabilities in the CNS and retina. However, subsequent studies have revealed that these capsid variants, such as AAV.PHP.B and AAV.PHP.eB,[84,85] produce remarkable CNS transduction in mice, but perform poorly in NHPs.[86,87] This discrepancy is due at least in part to the vectors interacting with receptors that are unique to mice such as Ly6a, which is expressed only in mouse brain endothelial cells.[88,89] Consequently, these variants exhibit poor translation to humans, significantly limiting their clinical applications. While AAV2.7m8 demonstrates impressive photoreceptor transduction in mice via intravitreal injection, its efficacy in NHPs is restricted, with retinal transduction observed only in the macular and peripheral areas, and very limited photoreceptor transduction.[67] This again highlights the challenge of cross-species barriers in AAV vector development. As a result, recent studies have shifted toward screening in NHPs, given their closer genetic and biological similarity to humans. A recent study reported a novel artificial intelligence (AI)-designed AAV capsid developed using *in vivo* data from NHP eyes injected with multiplexed AAV capsid libraries, which showed an 80-fold improvement in retinal transduction over AAV2 and a 2.6-fold increase compared to an externally engineered capsid, with low ocular inflammation in NHP models.[90] Similarly, another study demonstrated that the novel AI-engineered AAV capsids AVT260 and AVT239 achieved significantly higher retinal transduction efficiency via intravitreal injection in NHPs with 5- and 2-fold improvements over the benchmark AAV2.7m8, respectively.[91]

ML-based design

AI, specifically ML, is an emerging data-driven technology in the field of capsid engineering that is being developed rapidly.[92,93] In a pioneering work using ML to discover novel AAV capsids,[94] the entire AAV2 capsid fitness landscape was scrutinized by characterizing all single-codon substitutions, insertions, and deletions, which uncovered critical information regarding both surface-exposed and buried residues. Informed with this critical information, ML was used to design variants containing multiple mutations by sampling mutations at each residue proportional to their measured delivery effect on the target tissue, resulting in the need to create far fewer variants to screen than random mutagenesis approaches. Furthermore, the same group applied deep learning to design more diverse and viable AAV2 capsid variants by focusing on positions 561–588 and accurately predicted capsid viability across diverse variants with limited data.[95] This strategy provides new insights on engineering other regions of capsid gene aside from the classic positions, such as N587, thereby yielding more diverse variants with potential applications for future AAV development. A recent study reported that ML-guided AAV capsid design produced capsid variants with up to 80 times more efficient transduction of the neural retina, including rods, cones, microglia, and retinal ganglion cells relative to rAAV2 in NHPs after intravitreal injection.[96] Subsequently, a low-bias, high-diversity library was created and underwent *in vivo* screening in mice and *in vitro* screening in human cells for various traits that are desirable in gene therapy vectors, such as increased packaging capacity and specific tissue tropism. These data were then used to train ML models, which effectively predicted the biodistribution of AAV capsid variants in macaques.[97] The success of these predictions demonstrates the potential of ML models to inform capsid design and guide AAV development for future NHP studies and first-in-human clinical trials.

rAAV IN CLINICAL APPLICATIONS FOR OCULAR DISEASES

Ocular diseases are attractive therapeutic targets at the forefront of rAAV-based gene therapy; as of December 2023, there are 78 ongoing or completed clinical trials involving rAAV to treat ocular diseases (Figure 2 and Table S1). Of these, 15% are neovascular AMD followed by 14% being related to LCA 2 (LCA2). More than 60% of the analyzed clinical trials are still in the early stage (phase 1 and phase 1/2) focusing on safety, while 15% of these trials have advanced to phase 3, indicating a notable increase compared to a previous analysis conducted in 2019.[74] Among these trials, rAAV2 is clearly the predominant serotype being used.

IRDs

IRDs are a group of genetically heterogeneous disorders that lead to progressive vision loss and ultimately blindness. As there is currently no treatment for IRDs, but rAAV-based gene therapy has emerged as a promising approach to restore retinal function either by delivering a functional copy of a defective gene, introducing a therapeutic gene, or editing genomic DNA to correct the mutation. Several of the ongoing or completed clinical trials using rAAVs have been conducted to treat monogenic IRDs.

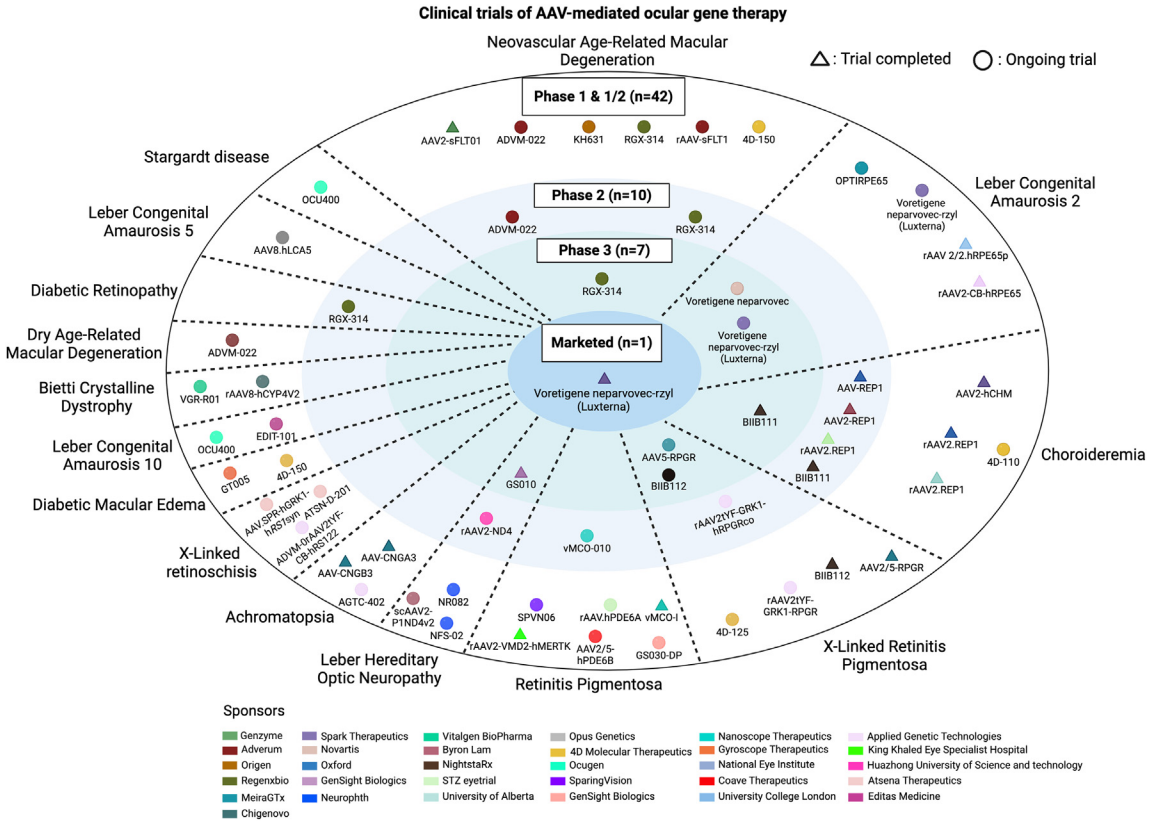

Figure 2. Analysis of rAAV-based gene therapy clinical trials for ocular disorders

A total of 78 clinical trials (both ongoing and completed) related to rAAV-based ocular gene therapy were identified (ClinicalTrials.gov; Table S1). The diagram specifically encompasses distinct investigational drugs belonging to the same phase within identical disease categories (i.e., if a particular investigational drug is involved in multiple trials within the same phase, it is considered only once). As such, it is important to note that this diagram provides a selective view and does not account for the total number of clinical trials of rAAV-based ocular gene therapy.

LCA2

LCA2 is a severe IRD caused by mutations in the *RPE65* gene, which is crucial for the visual cycle. Early work from multiple phase 1/2 clinical studies by different groups demonstrated the safety and therapeutic efficacy of AAV-based gene therapy for LCA2, laying the foundation for further developments in human studies.[98–101] The most significant advancement in AAV-based therapy for LCA2 has been the development of voretigene neparvovec-rzyl, an AAV2 vector carrying a functional copy of the *RPE65* gene. Voretigene neparvovec-rzyl was the first FDA-approved gene therapy for a genetic disease in the United States.[102] A landmark phase 3 study involving 31 participants with *RPE65*-associated IRD demonstrated that voretigene neparvovec significantly improved functional vision, with a mean change of 1.8 light levels in a multiluminance mobility test performance at 1 year post delivery in the intervention group compared to 0.2 in the control group (p = 0.0013) and 65% of treated participants achieving maximum improvement at the lowest light level. Importantly, there were no serious product-related adverse events reported.[103] These improvements were consistently maintained in long-term follow-up studies spanning the subsequent 3–4 years.[104,105]

Choroideremia

Choroideremia (CHM) is an X-linked recessive dystrophy of the photoreceptors, RPE, and choroid caused by mutation of the *CHM* gene. The first gene therapy clinical trial for CHM using rAAV2 encoding Rab escort protein 1 (*REP1*) was conducted in 2011, followed by several other multicenter clinical trials worldwide. In this first clinical trial, six patients had a mean gain of 3.8 letters in visual acuity within 6 months after the subretinal injection and the increase in retinal sensitivity in the treated eyes was correlated with the vector dose administered per square millimeter of the surviving retina.[106] In a 3.5-year follow-up study, the improvements were sustained in two of six patients, while visual acuity in the control eyes was better than that in the treated eyes at baseline.[107] In another study, 12 patients gained a median of 5.5 letters above their baseline in best-corrected visual acuity (BCVA), while two patients developed complications and lost 15 and 14 letters.[108] Three other clinical trials using the same vectors at a higher dose reported various outcomes in terms of visual gain, from 0 ± 7.5 letters to 2 ± 4 letters in 2 years following gene therapy.[109–111] In another clinical trial, no significant improvements of visual acuity were observed by 2 years post-treatment, while

adverse effects such as subconjunctival hemorrhage and changed intraocular pressure were reported in two patients.[112] Despite the mixed clinical outcomes, clinical trials for CHM advanced to a randomized, masked, phase 3 gene therapy study known as STAR to evaluate timrepigene emparvovec (BIIB111/AAV2-*REP1*).[113] The study examined the safety and efficacy over a 12-month follow-up in adult males with CHM who were randomized to receive either a high-dose (1.0×10^{11} vg/eye; $n = 69$) or low-dose (1.0×10^{10} vg/eye; $n = 34$) subretinal injection of timrepigene emparvovec, compared to an untreated control group ($n = 66$). However, the trial did not achieve its primary endpoint of BCVA improvement. It showed that 3 of 65 participants (5%) in the high-dose group, 1 of 34 participants (3%) in the low-dose group, and 0 of 62 participants (0%) in the control group showed a 15-letter or greater Early Treatment Diabetic Retinopathy Study (ETDRS) improvement from baseline BCVA at 12 months, while safety results were consistent with previous studies.

Retinitis pigmentosa

Retinitis pigmentosa (RP) is a genetically heterogeneous group of IRDs resulting from mutations in more than 80 different genes and characterized by progressive photoreceptor degeneration, leading to vision loss and blindness.[114] Mutations in the RP GTPase regulator (*RPGR*) gene are responsible for the most common form of X-linked RP (XLRP).[115] XLRP primarily affects males and is characterized by progressively vision loss due to dysfunction in both photoreceptors and RPE cells.[116] Significant advancements have been made using AAV-based gene therapy strategies targeting *RPGR*, and multiple clinical trials are underway.[117–119] One of the most notable is a phase 1/2 study investigating an AAV8 vector delivering a codon-optimized version of *RPGR* (AAV8-*RPGR*). Preliminary results from this trial have shown promising safety and efficacy, with several participants experiencing improved visual acuity and increased retinal sensitivity. Importantly, the therapy has been well tolerated, with no dose-limiting toxicities reported.[117] A *post hoc* analysis of the XIRIUS gene therapy trial along with the XOLARIS natural history study for XLRP revealed that some participants had early and sustained retinal sensitivity and low-luminance visual acuity over 12 months after receiving subretinal injection of cotoretigene toliparvovec (BIIB112/rAAV8-*RPGR*).[120] However, a phase 2/3 XIRIUS trial failed to meet its primary endpoint of a 7-dB or greater improvement from baseline in 5 or more of the 16 central loci in the 10-2 grid assessed by microperimetry at 12 months after treatment. Other clinical trials are actively exploring different AAV vectors for *RPGR* gene therapy to enhance therapeutic outcomes for XLRP. One phase 1/2 trial (NCT03252847) of AAV5-hRKp.*RPGR* in males with *RPGR*-associated XLRP demonstrated that the treatment was safe and well tolerated, with no dose-limiting events and most adverse events being transient and related to the surgical procedure.[65] The therapy showed improvements in retinal sensitivity and functional vision compared to the control group at week 26 and similar trends observed at week 52, supporting further investigation in a phase 3 trial.

Autosomal-recessive RP caused by mutations in the nuclear receptor subfamily 2 group E member 3 (*NR2E3*) gene is characterized by progressive vision loss due to the degeneration of photoreceptors. NR2E3 plays a critical role in maintaining the balance between rod and cone photoreceptors, and mutations in this gene can lead to night blindness, loss of peripheral vision, and eventual central vision impairment. A phase 1/2 clinical trial of OCU400 (AAV5-h*NR2E3*) gene therapy for *NR2E3*-and rhodopsin (*RHO*)-associated RP enrolled 18 subjects and demonstrated relative safety at low and medium doses, with most adverse events resolving within weeks.[121] Efficacy results showed that 86% of *RHO*-associated RP subjects experienced stabilization or improvement in visual function, including up to a three-luminance level improvement in mobility testing, while no improvements were observed in patients with *NR2E3* mutations. Further testing is needed to validate these findings.

Phosphodiesterase 6B (*PDE6B*)-associated RP is caused by mutations in the *PDE6B* gene, which encodes the beta subunit of the rod photoreceptor cyclic guanosine monophosphate phosphodiesterase enzyme. Mutations in *PDE6B* lead to rod photoreceptor dysfunction and degeneration, resulting in progressive vision loss, beginning with night blindness and peripheral vision loss, and ultimately leading to complete blindness. The 12-month results of a phase 1/2 trial of CTx-*PDE6b*, an AAV5-based gene therapy that delivers functional *PDE6B* copy, demonstrated that the therapy was well tolerated in all 17 patients.[122] A subgroup of six patients with less advanced disease who received the higher dose showed significant improvements in visual function across multiple clinical endpoints, including BCVA, visual field, and microperimetry, as well as favorable retinal anatomical changes on OCT, supporting further clinical development.

Another example of an IRD for which gene therapy strategies are being developed is loss-of-function mutations in the MER tyrosine kinase (*MERTK*) gene, a key element involved in phagocytosis in the RPE, results in photoreceptor degeneration and ultimately RP.[123] This autosomal-recessive disease phenotype constitutes approximately 3% of RP cases. A phase I study assessed the safety of subretinal administration of rAAV2-VMD2-h*MERTK* in six patients, with doses of 150 μL and 450 μL administered. Over a 2-year follow-up, the treatment demonstrated an acceptable safety profile, with no major complications attributed to the gene vector, although some patients experienced minor ocular issues. Three patients showed improved visual acuity after surgery, but two of these patients lost the improvement by the 2-year mark, suggesting potential, but variable clinical benefits.[123]

Biallelic mutations in the retinaldehyde binding protein 1 (*RLBP1*) gene cause rare autosomal recessive retinal dystrophies, including RP and other related disorders, characterized by night blindness, prolonged dark adaptation, and progressive vision loss. With approximately 160 cases that have primarily been reported in Sweden and Canada, the prognosis remains poor as the disease progression is not well understood due to the lack of natural history studies.[124] A clinical trial for CPK-850, an AAV-based gene therapy, is currently undergoing first-in-human, proof-of-concept testing. This study aims to establish the maximum tolerated dose of CPK-850 via a single

subretinal injection and assess its safety and potential efficacy in improving visual function in patients with *RLBP1*-associated RP. As of this writing, no definitive results have been published.

In addition to gene replacement or editing approaches, other strategies that work independent of the genetic mutation have been investigated to treat RP, such as optogenetics. Optogenetics involve the introduction of light-sensitive proteins into surviving retinal cells, such as bipolar or ganglion cells, rendering them responsive to light stimuli.[125–128] By using external light sources, these modified cells can be activated, potentially restoring vision in individuals who have photoreceptor degeneration. In a pioneering clinical trial involving a single patient with RP receiving a vector delivering optogenetic gene, GS030-DP (rAAV2.7m8-CAG-ChrimsonR-*tdTomato*), and light stimulation via engineered goggles, the patient gained the abilities to perceive, locate, count, and touch various objects, with multichannel electroencephalographic recordings detecting object-related activity above the visual cortex using the treated eye.[125] Recent developments include the initial findings from a phase 2b clinical trial, where intravitreal injection of MCO-010, an opsin encoded in rAAV2, was administered to RP patients to specifically target ON bipolar cells. Encouragingly, this intervention resulted in vision improvement and no serious ocular or systemic adverse events were reported. These outcomes underscore the potential of optogenetic strategies as a novel and effective therapeutic avenue for individuals with vision loss due to RP, which could be adapted to treat other ocular disorders and causes of blindness.[129]

Leber hereditary optic neuropathy

Leber hereditary optic neuropathy (LHON) is a maternally inherited mitochondrial disorder that causes progressive vision loss and blindness, typically in young males.[130] Three primary point mutations are responsible for approximately 90% of patients with LHON, which are located in the *MT-ND1*, *MT-ND4*, and *MT-ND6* mitochondrial genes. The mutations result in retinal ganglion cells undergoing apoptosis, leading to severe visual loss.[131] An early gene therapy trial assessed intravitreal injection of rAAV2 encoding a codon-optimized human *MT-ND4* gene in patients with a G to A mutation in the *MT-ND4* gene. The construct contains the CMV promoter and *cis*-acting elements of the human cytochrome *c* oxidase 10 (*COX10*) gene with a mitochondrial targeting sequencing at the 5′ end, leading to mRNA translation and co-translocation of the protein into mitochondria.[132] In a phase 1 clinical trial for treating LHON, 28 patients received unilateral intravitreal scAAV2(Y444,500,730F)-P1ND4v2 gene therapy across three vectors doses (5×10^9, 2.46×10^{10}, and 1×10^{11} vg/eye.).[133,134] Visual outcomes were mixed, with BCVA showing modest improvements in some eyes, though significant retinal thinning and no substantial change in steady-state pattern electroretinogram amplitude were noted. Incident uveitis was reported in 29% of cases, predominantly in higher dose groups, with no long-term vision loss. In a phase 3 clinical trial, REVERSE, 37 LHON patients with vision loss within the past 6–12 months were treated with intravitreal injection of lenadogene nolparvovec (rAAV2/2-*ND4*).[135] By week 96, 25 subjects demonstrated clinically relevant recovery in BCVA from baseline in at least one eye, while 29 subjects exhibited improved vision in both eyes with no serious adverse effects reported. Similarly, another phase 3 clinical trial, RESCUE, 38 participants receiving intravitreal injection of lenadogene nolparvovec showed comparable visual outcomes in the both injected and uninjected eyes in a 96-week follow-up.[136] A long-term gene therapy study using rAAV2/2-*ND4* in nine patients with LHON also showed varying degrees of visual recovery, particularly in younger patients, within 1–2 years of disease onset.[137] These studies underscore the challenges in treating chronic LHON and the need for more controlled trials to determine the true efficacy of gene therapy.[138] The variability in results from existing LHON gene therapy trials likely arises at least in part from differences in patient populations, disease stages at treatment initiation, and trial designs. Therefore, larger, randomized controlled trials are necessary to comprehensively evaluate the safety and effectiveness of gene therapy for LHON.

Achromatopsia

Achromatopsia, known as total color blindness, is an autosomal recessively inherited IRD that affects the cone photoreceptors which are responsible for high-acuity daylight vision. Up to 90% of patients with achromatopsia carry mutations in either the cyclic nucleotide gated channel subunit alpha 3 (*CNGA3*) or beta 3 (*CNGB3*) genes, which encode subunits of the cyclic nucleotide-gated (CNG) channels that are essential for phototransduction in cone photoreceptor cells.[139] Gene replacement therapy applying rAAV encoding either of the two genes has demonstrated successful proof-of-concept in various animal models, indicating recovery of cone photoreceptor function.[140–142] In a clinical trial assessing AAV8.*CNGA3* gene therapy for *CNGA3*-linked achromatopsia, nine patients received a single subretinal injection of varying doses and were followed for 12 months. The treatment showed no substantial safety issues and all treated eyes exhibited some improvement in cone function, with a mean visual acuity gain of 2.9 letters and an average contrast sensitivity increase of 0.33 log units, indicating potential benefits of the therapy for this condition.[143] In a study on cortical plasticity after gene therapy for *CNGA3*-associated achromatopsia, two adult patients showed minor improvements, including reduced photoaversion and marginal acuity gains, with one gaining the ability to detect red color. Functional magnetic resonance imaging analysis revealed a decrease in the receptive field size in the treated eyes, indicating some cortical adaptation. However, no activation of color-specific cortical regions was observed after treatment, suggesting either incomplete recovery of cone function or challenges in adult cortical processing of new cone-derived input.[144] A novel multimodal approach demonstrated that gene therapy can activate dormant cone-mediated pathways in children aged 10–15 years with *CNGA3*- and *CNGB3*-associated achromatopsia. In a study of four patients treated with subretinal rAAV vectors expressing a codon-optimized human CNGA3 gene under the PR1.7 cone opsin promoter (rAAV2-tYF-PR1.7-h*CNGA*co), two showed significant improvements in cone-mediated visual function and retinotopic patterns in the visual cortex after treatment, which were not observed in the untreated controls. This evidence suggests that gene therapy can induce neural plasticity and restore some cone function during childhood.[145]

LCA10

LCA10 is an autosomal-recessive ciliopathy, leading to severe visual impairment or blindness in early childhood.[146] The most frequent mutation (c.2991+1655A>G) found in patients with LCA10 is an intronic mutation in the centrosomal protein 290 (*CEP290*) gene that results in a cryptic splice donor site. CEP290 is a large protein located in the ciliary transition zone of cone and rod photoreceptors and is crucial for forming and stabilizing primary cilia. It regulates protein trafficking between the photoreceptor inner and outer segments, which is essential for phototransduction and photoreceptor function. While standard gene replacement therapy is challenging for treating LCA10 due to the large size of *CEP290*, gene editing using the CRISPR-Cas9 system represents a promising approach to correct the splice mutation. One study delivered a dual rAAVs carrying a self-limiting CRISPR-Cas9 system and managed to delete an intronic fragment of the *CEP290* in the mouse retina.[147] A landmark clinical trial to test the ability of a CRISPR-Cas9 system (EDIT-101) delivered by rAAV through subretinal injection to remove *CEP290* mutations in retinal cells of patients with LCA10 was started in 2020.[148] This phase 1/2, open-label, single-ascending-dose study enrolled 14 participants aged 3 years or older with *CEP290*-associated LCA10 caused by the IVS26 variant. The primary outcome was safety, and no serious adverse events or dose-limiting toxic effects were observed. Among the 12 adults and 2 children treated, 64% experienced a meaningful improvement in key secondary outcomes, including BCVA, retinal sensitivity, and mobility test scores. Additionally, six participants showed improvement in vision-related quality of life. The findings support further research into *in vivo* CRISPR-Cas9 gene editing for treating IRDs due to the IVS26 variant.[149]

X-linked retinoschisis

X-linked retinoschisis (XLRS) is a developmental IRD that results in retinal cavities, synaptic dysfunction, reduced visual acuity, susceptibility to retinal detachment, and ultimately impairs vision, which typically beginning in young males and progresses with age. It is caused by mutations in the retinoschisin 1 (*RS1*) gene, which plays a critical role in cell adhesion during the normal development and maintenance of retinal structure. Gene therapy for XLRS aims to deliver a functional copy of the *RS1* gene to the affected retinal cells, and several clinical trials have been conducted to evaluate the safety and efficacy of putative treatments. In a phase 1/2a trial, nine male participants with XLRS received intravitreal AAV8-*RS1* at escalating doses (1.0×10^9, 1.0×10^{10}, or 1.0×10^{11} vg/eye). The therapy was generally well tolerated, with dose-related inflammation effectively managed using corticosteroids. While no antibodies against RS1 were detected, systemic antibodies against AAV8 increased with higher doses and retinal cavities transiently closed in one participant, leading to further exploration of dosing and immunosuppressive regimens to optimize safety and efficacy.[150]

ATSN-201, an AAV.SPR-hGRK1-h*RS1*syn gene therapy, is being investigated for treating XLRS. It uses a novel AAV.SPR capsid specifically designed to efficiently deliver the *RS1* gene to the retina, targeting the central retina where schisis cavities commonly occur. The AAV.SPR capsid spreads laterally from the subretinal injection site, reaching the fovea without direct injection into this delicate area, minimizing the risk of retinal damage. In the ongoing phase 1/2 LIGHTHOUSE study, ATSN-201 has shown a favorable safety profile, with no serious adverse events reported, and two out of three patients in the first cohort demonstrated extensive resolution of retinal schisis from 8 weeks after dosing, continuing through week 24.

Bietti crystalline dystrophy

Bietti crystalline dystrophy (BCD) is a progressive, rare, autosomal-recessive IRD characterized by numerous clear glistening yellow-white crystalline deposits forming mainly in the RPE layer. BCD is attributed to loss-of-function mutations in the cytochrome P450 family 4 subfamily V polypeptide 2 (*CYP4V2*) gene, which is associated with the metabolism of fatty acids and steroids through ω-hydroxylation.[151] Gene replacement strategies have been attempted in both *in vitro*[152] and *in vivo* models,[153] which have successfully expressed a functional *CYP4V2* gene delivered by rAAV2. In a trial evaluating NGGT001, an AAV-based gene therapy for BCD, patients experienced a mean visual gain of 12.8 ± 3.7 ETDRS letters at 9 months after treatment, with 46% of patients gaining more than 15 letters. This therapy was also well tolerated, with mild, self-resolving adverse events related to the subretinal injection procedure and no intraocular inflammation observed. In a separate trial involving rAAV2/8-h*CYP4V2* gene therapy for BCD, 77.8% of treated eyes showed improved BCVA by day 180 with an average gain of 9.0 ± 10.8 letters, and by day 365, 80% of treated eyes demonstrated further improvement with an average gain of 11.0 ± 10.6 letters. The trial reported 73 treatment-emergent adverse events, which were mostly mild to moderate with no serious treatment-related adverse events or immune toxicities, further supporting the safety and potential efficacy of the therapy.[154] Notably, a slight improvement in BCVA was also observed in the untreated contralateral eye. This phenomenon is consistent with findings from several previous IRD gene therapy clinical trials and may be attributed to factors such as visual cortex activation, brain plasticity, or vector shedding into the contralateral eye.

Common acquired or multifactorial retinal conditions

While many clinical trial results are promising, there are a small number of people with each rare monogenic IRD, which contributes to the significant costs associated with gene therapy. In contrast, targeting common acquired retinal conditions could have the potential to positively impact a significant number of patients and make the access to therapies more affordable.

AMD

AMD is characterized by a progressive retinal geographic atrophy (GA) and/or neovascularization in its advanced stages and is the major cause of blindness in developed countries. The mainstay treatment for neovascular AMD is to target vascular endothelial growth factor (VEGF), a key angiogenic factor involved in the pathogenesis of retinal neovascularization. Although anti-VEGF treatments have mitigated disease progression and enhanced visual function in many patients with AMD, this treatment requires a monthly

injection, which poses a substantial treatment burden. In addition, a considerable proportion of patients are not responsive to anti-VEGF reagents.[155] Gene addition therapy through rAAV delivery has the potential to provide an enduring, endogenous production of biological factors to treat AMD, thereby bypassing many limitations associated with repeated ocular injections. Consequently, an increasing number of clinical trials are leveraging rAAV to deliver specific genes, targeting both GA and neovascular AMD.

GA is a chronic and progressive degeneration of the macula, leading to the formation of distinct and well-defined areas of RPE and photoreceptor atrophy, a feature of advanced dry AMD.[156] GT005 is a one-time gene therapy using rAAV2 encoding the complement factor I (*CFI*) gene, designed for GA resulting from AMD through subretinal injection. The phase 1/2 FOCUS study of GT005, which aimed to treat GA in dry AMD, demonstrated that the therapy was generally well tolerated and showed early signs of potentially slowing GA progression in a small group of patients. Building on these initial findings, two larger phase 2 trials, EXPLORE and HORIZON, were launched to assess the efficacy of GT005 in a broader population, including patients with a high-risk *CFI* variant. Early results from these trials suggest that GT005 could reduce lesion progression, particularly in genetically predisposed patients. However, despite these promising outcomes, the development of GT005 was ultimately discontinued after further evaluation indicated that the therapy did not meet the necessary benefit-risk profile for continued development. Similarly, OCU410 utilizes an AAV vector to subretinally deliver the RAR-related orphan receptor A (*RORA*) gene involved in lipid metabolism and inflammation as a treatment for dry AMD. Currently, OCU410 is under evaluation in the phase 1/2 ArMaDa clinical trial, an open-label, dose-ranging study. The trial involves three dose levels—low (2.5×10^{10} vg/mL), medium (5×10^{10} vg/mL), and high (1.5×10^{11} vg/mL)—and has completed dosing across all cohorts. The primary focus of the trial is on safety, while secondary objectives include assessing the therapy's efficacy in slowing the progression of GA. Long-term outcomes on vision preservation and disease progression are being closely monitored.

Another gene addition therapy for neovascular AMD has used rAAV2 to deliver a genetic payload of *sFlt-1* via intravitreal and subretinal administration.[157–160] This therapeutic protein is a chimeric VEGF inhibitory protein comprising domain 2 of Flt-1 (VEGF receptor-1) fused with the Fc fragment of the human immunoglobulin G1 heavy chain.[161] In a phase 1 dose escalation clinical trial, intravitreal injection of rAAV2-*sFlt*-1 found that the treatment at various doses was safe and well tolerated in a 52-week follow-up, while the expression level of sFlt-1 and the treatment efficacy was variable.[162] This variability was likely due to the presence of anti-permeability and anti-AAV2 serum antibodies. In a combined phase 1 and phase 2a randomized controlled trials, subretinal injection of the same vectors was well tolerated, especially among the elderly.[163] Notably, although the study did not primarily focus on visual improvements, significant enhancements were not observed.

RGX-314 utilizes rAAV8 to encode anti-VEGF monoclonal antibody fragments and was developed to target neovascular AMD via subretinal injection.[164] Initial assessment from the phase 1 clinical trial ASCENT demonstrated the treatment was safe and well-tolerated in patients. The efficacy of the treatment was found to be dose dependent, with some patients remaining free from additional anti-VEGF injections for up to 18 months.

ADVM-022 is also a gene therapy that was developed for neovascular AMD via intravitreal injection, which uses the engineered 7m8 vector encoding the cDNA for the aflibercept protein, which is a soluble protein decoy for VEGF. In the phase 1 clinical trial, OPTIC, ADVM-022 showed impressive efficacy over 92 weeks of follow-up. The study found that more than 80% of patients who received the therapy did not require any additional supplemental anti-VEGF injections. This not only highlights the safety profile of ADVM-022 but also suggests a sustained positive impact, indicating a potentially transformative advancement in the treatment landscape for neovascular AMD.[165]

4D-150 is a gene therapy for wet AMD and DME, utilizing the R100 AAV vector—an engineered capsid that efficiently penetrates the internal limiting membrane, transduces the entire retina, and achieves strong transgene expression through intravitreal delivery.[166] 4D-150 delivers a dual transgene payload that expresses aflibercept and a VEGF-C inhibitory RNAi, targeting four angiogenic factors: VEGF A, B, and C and placental growth factor. In the phase 2 PRISM trial, 51 wet AMD patients with severe disease and a high treatment burden (averaging 10 anti-VEGF injections in the prior 12 months) were randomized to receive either a high-dose (3×10^{10} vg/eye) or low dose ($1 \times$-10^{10} vg/eye) of 4D-150, or aflibercept. The therapy showed a favorable safety profile with no significant intraocular inflammation and successful steroid tapering by 26 weeks. The high-dose group achieved an 89% reduction in annualized anti-VEGF injection rates, with 84% requiring no or one supplemental injection and 63% remaining injection free at 24 weeks. Visual acuity and central subfield thickness were stable compared to the aflibercept control group. These encouraging results are advancing the therapy toward a phase 3 trial, which is expected to start in the first quarter of 2025.

Diabetic retinopathy

Diabetic retinopathy (DR) is a common complication of diabetes mellitus and a leading cause of blindness in developed countries and includes proliferative DR (PDR) and DME.[167] PDR is characterized by the abnormal blood vessel growth in the retina, while DME features the retinal vascular leakage and edema in the macula. Current clinical trials of rAAV-based gene therapy for DR mainly focuses on the endogenous production of therapeutic proteins, with many targeting VEGF.[168]

Similar to neovascular AMD, RGX-314 is also being developed as a potential one-time gene therapy for patients with DR. In the ongoing phase 2 ALTITUDE trial, RGX-314 was administered via suprachoroidal delivery and demonstrated a strong safety profile in 50 patients across two dose levels tested, with no drug-related serious adverse

events reported.[169] At 1 year after treatment, 70.8% of patients treated at the higher dose achieved an improvement of at least 1 step on the Diabetic Retinopathy Severity Scale (DRSS), compared to 25% in the control group. Additionally, the therapy reduced the risk of vision-threatening events by 89%, with 0% of treated patients experiencing a worsening of 2 or more steps on the DRSS compared to 37.5% in the control group. The application of RGX-314 for DME is being further developed. Moreover, safety and efficiency of intravitreal delivery of ADVM-022 was also assessed in patients with DME (INFINITY trial). However, a patient experienced an unexpected serious adverse reaction of hypotony in an eye treated with the high dose (6.0×10^{11} vg/eye).[170] Five out of 12 patients receiving the high dose experienced additional adverse events during the 16–32 weeks after ADVM-022 administration, including rapid, clinically relevant decreases in intraocular pressure, which were refractory to steroids. This unexpected serious adverse reaction occurred at 30 weeks after randomization in the highest dose group, prompting the sponsor to suspend the trial while conducting ongoing analyses. Despite this, earlier trials, such as the OPTIC study for wet AMD, showed significant reductions in treatment burden with ADVM-022, which was generally well tolerated. Importantly, the study revealed significant variations among the DME and neovascular AMD patients and between the lower (2.0×10^{11} vg/eye) and higher (6.0×10^{11} vg/eye) dosage levels. The comprehensive analysis postulated that wet AMD and DME exhibit distinct pathophysiological origins and associated risk factors. Although the exact cause of the dose-limiting toxicity remains unknown, DME patients commonly present with multiple underlying comorbidities, including severe vascular disease, which may contribute to inflammatory factors leading to heightened vascular permeability and disruption of the blood-ocular barrier. Indeed, a study uncovered a dosage-dependent impact of the 7m8 vector encoding an anti-VEGF gene on vascular sheathing pathology in the mouse retina resembling vasculitis in humans, a vascular inflammation typically accompanied by immune cell infiltrates.[171] This study suggested that the serious adverse event observed in the patient receiving ADVM-022 may result from a synergistic effect of heightened inflammation due to the underlying disease and the inhibition of VEGF function. Over time, this dual impact could have intensified the inflammation associated with the disease.[172]

CHALLENGES OF rAAV-BASED OCULAR GENE THERAPY

Although substantial progress has been achieved, several challenges remain in the development of rAAV, all of which fundamentally affect the safety and efficacy of rAAV-based ocular gene therapy (Figure 3).

Immunogenicity and inflammation induced by rAAV

Many factors can affect the efficacy of rAAV transduction in the eye, several of which are related to the host immune system. Although the eye is a relatively immune-privileged organ, inflammation in ocular gene therapy is common and is often associated with reduced transduction efficacy, lower cell viability, and higher immunotoxicity.[173] Pre-existing NAbs in the serum have been a major concern for systemic administration of rAAV. Retina-targeting rAAVs are also susceptible to NAb-mediated neutralization, mostly when delivered by intravitreal injection, although the NAb titer in the vitreous humor is significantly lower than that in the serum.[174] Patients with intact blood-retinal barrier (BRB) tend to have very low levels of NAb in the vitreous humor. In patients with a disrupted BRB, the level of NAb in the vitreous humor is positively associated with the level in the serum. Intravitreal injection of rAAV can increase systemic NAb production against AAV and ocular delivery of vectors to NHPs with high serum NAb titers displayed weak or no transgene expression.[175] Interestingly, the NAb titer in the vitreous fluid were equal in both eyes, regardless of whether NHPs received injections in one or both eyes.[175] Since sampling vitreous fluid is clinically challenging, assessment of pre-existing NAbs in the serum and the integrity of the BRB may be a necessary alternative step before retinal gene therapy.

To minimize NAb neutralization, novel retinal-targeting capsids are being engineered to replace wtAAV serotypes, such as AAV2 and AAV8.[176,177] A recent study of the murine eye demonstrated that intravitreally injected antigens drain through lymphatic vasculatures in the optic nerve sheath into deep cervical lymph nodes, which is important for the development of antigen-specific $CD8^+$ T cells in the retina and the CNS.[178] Stimulation of lymphatic drainage by VEGFC further suppressed AAV-vectored retinal transduction, while inhibition of VEGFC signaling by sVEGFR3 allowed repeated AAV dosing in the retina. While this study did not assess NAb titer in the vitreous fluid, it is likely that anti-AAV NAb development is impaired by temporary inhibition of ocular lymphatics.

Clinically, ocular inflammation related to rAAV treatment is common in patients receiving either subretinal or intravitreal injections. The inflammation is dose-dependent but varies significantly across preclinical and clinical studies.[173] Factors like surgical techniques, administration routes, rAAV dose, vector preparation, promoter sequence, transgene toxicity, and immunosuppression before surgery all contribute to the extent of inflammation. Intravitreal injection, which exposes AAV particles directly to immune cells in the vitreous and retina, carries a higher risk of immune activation compared to subretinal injection. In NHPs, intravitreal dosing of AAV led to transient cellular inflammation in the aqueous and sustained inflammation in the vitreous, both increasing with dose.[179] Reducing the capsid dose by removing empty AAV capsids reduced inflammation and improved transduction. IVT injections also led to systemic production of NAbs, although cytokine levels and retinal inflammation were minimal at 3 months after injection. A clinical trial with 15 patients with LHON found that systemic immune responses to intravitreal injection of rAAV2-ND4 were transient and not linked to adverse inflammation.[180] Subretinal injections, being more immune privileged, typically cause less inflammation, as seen in a trial for LCA2 patients with *RPE65* mutations, where inflammation resolved with steroids after 14 days.[181] However, in a choroideremia trial using subretinal injection of rAAV2.REP1, one patient developed significant vitritis and retinitis, possibly due to vector reflux into the vitreous, requiring an extended immunosuppression regimen.[182]

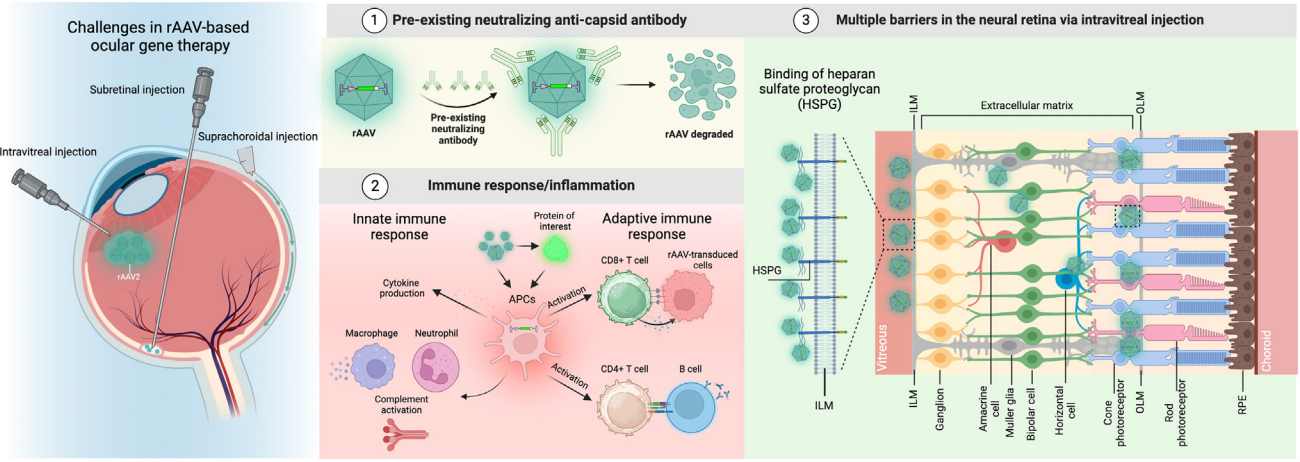

Figure 3. Challenges in rAAV-based ocular gene therapy

First, pre-existing antibodies against rAAVs can prevent cell/tissue transduction following intravitreal administration. Second, ocularly delivered rAAVs can induce both innate and adaptive immune responses in the eye, resulting in severe inflammation and significantly affecting therapeutic efficacy and safety. Third, rAAV, specifically rAAV2, molecules delivered through intravitreal injection are not able to penetrate the outer retina where photoreceptors and RPE are located, both of which are degenerated in most IRDs. This limitation is primarily due to binding between rAAV2 and HSPG. HSPG is the primary receptor that rAAV2 binds to and is enriched in both ILM and outer limiting membrane (OLM), as well as other barriers, such as the extracellular matrix in the retina.

It has been long thought that the genetic cargo of rAAV, in particular the CpG-containing ITRs, may act as triggers of innate immune responses that promote inflammation. The unmethylated CpG receptor Toll-like receptor 9 (TLR9) is critical for B cell activation and antibody production[183,184] and TLR9 stimulation by CpG ODN significantly promoted OT-I (SIINFEKL-specific) $CD8^+$ T cell responses against mice transduced with AAV8-SIINFEKL.[185] To mitigate this response, an AAV vector with CpG-free ITRs was made, which demonstrated comparable transduction efficiency with vectors containing wt ITRs but had markedly reduced vector yield during production.[186] Another strategy is to incorporate TLR9 inhibitory sequences within the vector.[187–189] The telomer-derived ODN TTAGGG was inserted between the 5′ ITR and the transgene and led to better GFP expression and lower infiltration by total $CD3^+$ and $CD8^+$ T cells in the murine eye,[188] although its efficacy in larger animals including NHPs remains to be assessed. Of note, there is no clear evidence that other intracellular DNA receptors, including AIM2, STING, and those associated with the type I interferon pathway, participate in the recognition of rAAV genetic cargo.[190]

Limited cargo capacity of rAAV

The approximately 4.7-kb cargo capacity of rAAVs can be present limitations for certain applications, such as gene replacement strategies to treat Stargardt disease-associated mutations in *ABCA4* (cDNA length of approximately 6.8 kb). To overcome this size restriction, one possibility is to deliver the transgene through two rAAVs in which the oversized transgene is split between two discrete vectors. One vector harbors the promoter and the 5′ portion of the transgene, while another vector includes the 3′ portion of the transgene and the poly(A) tail. The two vectors contain an overlapping and highly recombinogenic fragment of the human placental alkaline phosphatase (*AP*) gene. Once co-delivered, intermolecular concatemerization takes place via overlapping elements or the vector ITRs, which is recombinogenic, ultimately forming the full-length desired transgene product at the mRNA level. In one study, a hybrid *ABCA4* dual vector system was developed and delivered a functional *ABCA4* transgene into the $ABCA4^{-/-}$ mouse model of Stargardt disease.[191] A similar study validated that the dual vector system enables expression of the full-length *ABCA4* transgene in $ABCA4^{-/-}$ mice.[192] However, the efficiency of transgene expression through the dual vector system has been found to be inconsistent. A study of a dual vector system also observed both full-length and small fragments of *ABCA4*.[193] One limiting step for transduction using two vectors is that the two complementary ITRs could form circular concatemers, resulting in expression of a partial length of the transgene and production of nonfunctional proteins.[194] Alternatively, researchers have explored the use of split inteins, natural polypeptides that are capable of mediating protein *trans*-splicing. This approach succeeded in reconstituting large proteins like ABCA4 or CEP290 in animal models by fragmenting them into two split intein-flanked polypeptides whose sequences are compatible with a single rAAV.[195,196] However, its efficacy and safety in humans remain uncertain. Despite split intein-mediated protein *trans*-splicing expands AAV vector capacity for large gene delivery, the residual non-mammalian inteins pose safety concerns. A study demonstrated that a modified *E. coli* dihydrofolate reductase degron effectively reduces intein levels after *trans*-splicing, ensuring both safety and efficacy in a mouse model of Stargardt disease, supporting its use in future gene therapies. To address the hurdle of oversized transgenes, studies have alternatively explored highly minimized versions of transgenes with large sizes such as *CEP290*. Two studies revealed that subretinal injections of rAAV8 encoding a mini*CEP290* gene or a functional *CEP290* fragment into a mouse

model of LCA led to significant improvements in photoreceptor survival, morphology, and function as compared to the controls.[197,198]

Targeting specificity

Achieving efficient targeting of the desired retinal cell types is critical for a successful ocular gene therapy. Many studies focused on cell-specific promoters in the expression cassette to regulate transgene expression in specific retinal cell types. In a head-to-head comparison study, five promoters were compared to assess their capability of maximizing transgene expression in retinal ganglion cells. The human synapsin promoter had nearly exclusive transgene expression in retinal ganglion cells compared to ubiquitous promoters, such as CBA, CMV, and short CMV early enhancer/chicken β-actin/short β-globin intron.[199] The interphotoreceptor retinoid binding protein promoter can direct transgene expression specifically in both rod and cone photoreceptors in dog and mouse retina.[200] However, this promoter is ineffective in driving transgene expression to cone photoreceptors in NHPs.[201] Several RPE-specific promoters such as *RPE65*, *VMD2* (or *BEST1*), and *RPGR* have been evaluated in rAAV.[202–204] The human *RPE65* promoter has been used in an rAAV2/2 to drive the human RPE65 expression in a clinical trial of gene replacement therapy for LCA2 via subretinal injection.[99] However, there were no clinically significant changes in visual acuity in three patients receiving the injection. The *VMD2* promoter has been demonstrated to drive robust transgene expression specifically in RPE cells in many rAAV-based animal studies.[205,206] A recent study compared various photoreceptor- and RPE-specific promoters to assess their effects on the safety of rAAV in retinal cells after subretinal injection.[207] Interestingly, the results showed that ubiquitous promoters, such as CMV, human ubiquitin C promoter, and CAG, and RPE-specific promoters, such as *VMD2*, were all toxic as assessed by morphology, inflammation, and physiology, while photoreceptor-specific promoters, such as human red opsin, human rhodopsin, human rhodopsin kinase, and mouse cone arrestin were not toxic. The potential reasons for promoter-related toxicity in AAV vectors include the high levels of transgene expression driven by broadly active promoters like CMV, human ubiquitin C, and CAG, which can trigger an innate immune response, possibly through the generation of double-stranded RNA or other immune-stimulatory sequences. In contrast, photoreceptor-specific promoters, which drive more targeted and lower levels of expression, did not induce toxicity, suggesting that the choice of promoter is crucial for minimizing adverse effects in gene therapy. Additionally, RPE cells and microglia might be particularly sensitive to the effects of these broadly active promoters, leading to inflammation and retinal damage.

Administration routes

There are three common administration routes to deliver rAAV to the retina in the clinical settings: subretinal, intravitreal and superchoroidal injections (Figure 4). Subretinal injection is a primary route for most current gene therapies for photoreceptor and RPE diseases. Despite its effective delivery of rAAV to the retina, subretinal injection has several limitations that affect the efficacy and safety of rAAV transduction, which are described above. Delivering viral vectors through intravitreal injection would maximize the delivery area in the retina, thus transducing more retinal cells and increasing the efficiency of gene therapy. However, there is a general consensus that rAAV2 does not show high efficiency to transduce photoreceptors and RPE cells through intravitreal injection. Once delivered into the vitreous, rAAV2 faces several barriers. These include dilution due to the relatively large volume of the eyeball of large animals (especially in NHPs, where 0.05–0.10 mL of vector is dispersed into approximately 2.5 mL of vitreous humor), strong binding to HSPG in the ILM, the long diffusion distance from the point of delivery to photoreceptors or RPE cells, the extracellular matrix between retinal cells, and proteasomal degradation.[208,209] Therefore, there are increasing efforts focusing on engineering novel capsids to enable outer retinal transduction via intravitreal injection. Additionally, several human and NHPs studies revealed that intravitreal injection of rAAV induces a significant humoral immune response, as assessed by measuring anti-AAV antibody levels in the serum. One study demonstrated that intravitreal injection of rAAV in one eye blocks transgene expression in the contralateral eye upon re-administration through the same route, suggesting the production and circulation of NAbs.[210] Despite the concept of ocular immune privilege, several clinical studies suggested clear inflammation in the intravitreally rAAV-treated eyes. A study compared the degree of inflammation between intravitreal and subretinal gene therapy and revealed that intravitreal route led to a stronger humoral response, while the subretinal route caused more inflammation in anterior and posterior segment than the intravitreal route.[211] However, it is important to note that this inflammation induced by intravitreal injection is often transient, dose dependent, and manageable with appropriate treatments, such as corticosteroids. The severity and occurrence of inflammation can vary based on the rAAV dose, serotype, and patient-specific factors.[70,104,212] Therefore, while there is a risk of inducing immune responses, it does not necessarily undermine the potential benefits of rAAV therapy, especially with careful management.

In addition to subretinal and intravitreal injection, researchers have also investigated suprachoroidal delivery, an administration option that is typically used for conditions affecting the choroid. This non-invasive route of administration can be delivered in an office setting and can provide greater surface area coverage of the injected fluid compared to subretinal injection.[213] However, unlike intravitreal or subretinal injections, which are delivered directly into immune-privileged compartments of the eye, suprachoroidal injections require vectors to traverse multiple tissue layers to reach the outer retina. This increases the risk of an immune response due to greater exposure to immune cells and extracellular matrix components along their path.[214] Currently, only two clinical trials have used this route to deliver a gene therapy drug, which were studies of RGX-314 to treat DR and neovascular AMD. The long-term efficacy and safety profile of these drug have not yet been reported.

Long-term safety and efficacy

Safety concerns associated with rAAV-based gene therapy are central to all clinical trials. As these therapies aim for long-lasting or

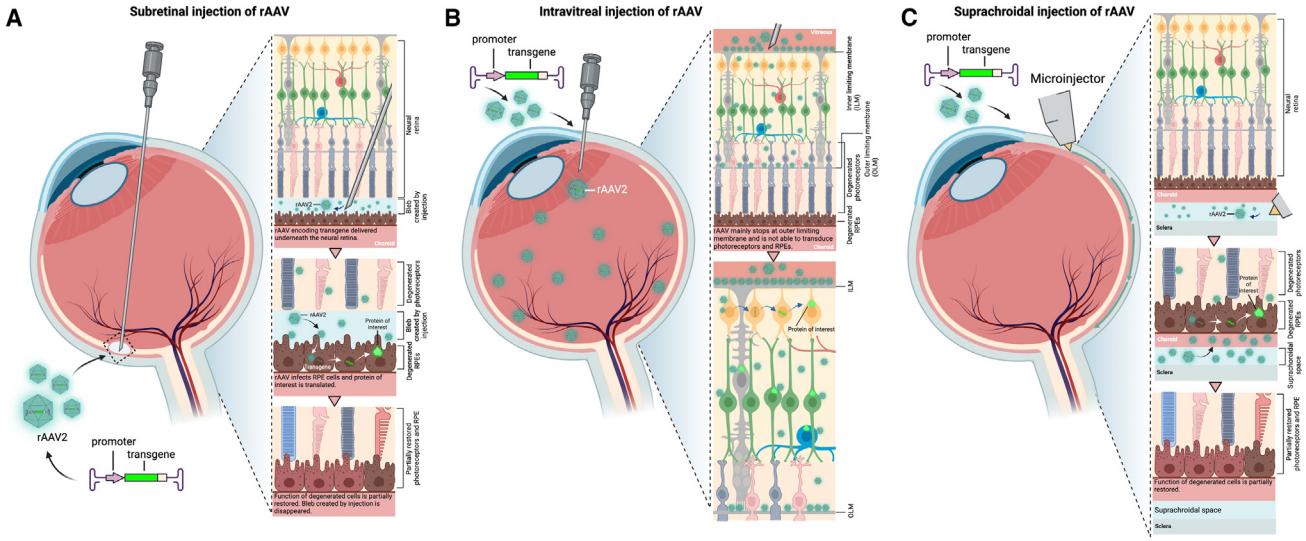

Figure 4. Schematic representation of subretinal, intravitreal, and suprachoroidal injection of rAAV

(A) Subretinal injection is a major administration route used in most current clinical trials for rAAV-based ocular gene therapy. rAAVs delivered through this route mainly transduce RPE cells and photoreceptors. (B) Intravitreal injection is a promising route of administration because it is easier to deliver and less invasive compared to subretinal injection. However, this administration route limits the rAAV transduction mainly to the inner retinal cells due to multiple barrier layers in the retina. (C) A microinjector-based suprachoroidal injection, which can be conducted in an office setting, facilitates the delivery of vectors to the suprachoroidal space, which is situated between the choroid and sclera. Once administered into the suprachoroidal space, rAAVs spread posteriorly and circumferentially.

permanent effects with a single treatment, careful attention is given to monitor long-term outcomes. Several clinical trials have revealed that rAAV-based ocular delivery of therapeutic genes led to wide variability in long-term visual improvements. A meta-analysis of six rAAV-based gene therapy clinical trials for *RPE65*-associated LCA (n = 164 eyes) revealed significant improvements in BCVA in the treated eye 1 year after treatment. Though variability in visual improvements was observed 2–3 years after treatment, these therapies still represent a significant step forward in addressing previously untreatable conditions.[215] In the case of voretigene neparvovec-rzyl, FDA documentation shows that approximately one-half of the treated patients met the threshold for meaningful improvement in vision, marking a substantial breakthrough for treating LCA.[216] The long-term effects of voretigene neparvovec-rzyl are still being investigated, with some studies suggesting potential limitations in its durability. One study highlighted that while initial improvements in visual function were observed in both patients and dog models with retinal degeneration, progressive photoreceptor loss continued over time despite the therapy.[217] This raises concerns about the sustained efficacy of voretigene neparvovec-rzyl and similar rAAV-based therapies, emphasizing the need for ongoing monitoring and follow-up studies to fully understand their long-term therapeutic potential.

FUTURE DIRECTIONS IN rAAV-BASED OCULAR GENE THERAPY

As rAAV-based ocular gene therapy continues to evolve, several key areas are emerging that promise to enhance the efficacy, safety, and applicability of these treatments. This section outlines these future directions, emphasizing innovations in vector design, the integration of gene editing technologies, and the challenges that must still be overcome.

Innovations in vector design

Significant advancements have been made in the design of rAAV capsids, with ongoing research aimed at creating vectors with enhanced specificity, reduced immunogenicity, and improved transduction efficiency. The development of the AAV2.7m8 variant marked a significant advancement, demonstrating superior photoreceptor transduction via intravitreal injection in rodents compared to AAV2. However, its efficacy in NHPs was limited, highlighting the need for further innovations.[67] Even with ML-assisted capsid design using data from NHP capsid screening, the resulting capsids reported in various studies only marginally improved photoreceptor transduction via intravitreal injection in NHPs,[90,91] indicating limited therapeutic potential for ocular gene therapy. This suggests that the current *in vivo* strategy in costly NHPs may not be the most effective approach for identifying capsid variants that achieve robust photoreceptor transduction in humans.

A recent study by Deverman's group adopted an *in vitro* screening approach by targeting the human transferrin receptor, which is enriched in human blood-brain barrier (BBB) and involved in transcytosis.[218] This led to the identification of an AAV9 capsid variant that effectively crosses the BBB and transduces CNS cells in NHPs. This approach provides a new strategy for capsid development by focusing on capsid variants that bind robustly to cellular receptors expressed in

human target cells or tissues, bypassing cross-species barriers and ensuring effective transduction in human cells. Such an approach could be adapted for capsid engineering in ocular gene therapy, targeting species-conserved receptors expressed in photoreceptors for intravitreal injection. To further enhance this process, a novel *in vitro* screening platform could be established to screen capsid libraries for HS binding affinity and selecting capsids with reduced HS binding. Combining these approaches in capsid screening could lead to the innovative discovery of capsids with both reduced HS binding and specific photoreceptor receptor binding, significantly enhancing photoreceptor transduction via intravitreal injection and transforming retinal gene therapy.

Gene editing technologies

The integration of rAAV with gene editing technologies, particularly CRISPR-Cas9, represents a frontier in ocular gene therapy that could revolutionize the treatment of IRDs. One notable example is the clinical trial of using rAAV to deliver CRISPR-Cas9 for the treatment of LCA10 caused by mutations in the *CEP290* gene (EDIT-101), which has reported encouraging outcomes in safety and efficacy (64% of participants experienced meaningful improvements in key secondary outcomes). Base editing and prime editing are emerging genome-editing techniques, each utilizing different mechanisms to precisely repair genetic mutations. While base editing uses a modified Cas9 enzyme to convert specific DNA bases without creating double-strand breaks, prime editing combines a Cas9 nickase with reverse transcriptase to introduce a wide range of genetic changes, including small insertions and deletions. Both techniques offer significant potential for correcting diverse genetic mutations with high precision. Due to the large size of base and prime editors exceeding AAV's packaging capacity, their application in rAAV-based ocular gene therapy is limited. However, one study successfully optimized a dual-AAV split-intein prime editor and delivered it via subretinal injection in an rd12 mouse model of LCA.[219] This method achieved up to 16% precise correction of the *RPE65* mutation, restoring RPE65 expression, improving retinal and visual functions, and preserving photoreceptors, without detectable off-target edits. This provides proof-of-principle success for the potential of prime editing for treating IRDs.

Compared to DNA editing, RNA editing offers several advantages, such as the ability to make transient and reversible modifications, which reduces the risk of permanent off-target effects in the genome.[220] One study demonstrated that AAV-delivered CRISPR-Cas13bt3, a novel RNA editor, effectively silenced *VEGFA* in human retinal organoids and humanized mouse models, achieving significant *VEGFA* mRNA reduction in hESC-derived RPE cells and specific knockdown of human *VEGFA* in mouse photoreceptor cells.[221]

Overcoming current limitations

To overcome the limitations of rAAV-based ocular gene therapy, several innovative strategies are being pursued. Capsid engineering is being used to develop less immunogenic AAV variants that can evade pre-existing NAbs and minimize immune responses, particularly in the context of intravitreal injections. Additionally, optimizing immunosuppression protocols and exploring alternative administration routes, such as suprachoroidal injections, are being considered to reduce inflammation and improve therapeutic outcomes. Addressing the limited cargo capacity of rAAV, researchers are developing dual vector systems and utilizing minimized or split transgenes to enhance the efficiency and consistency of transgene expression for conditions requiring large genes. Advances in vector design, including the use of overlapping elements and protein *trans*-splicing techniques, are proving effective in overcoming these challenges. Furthermore, refining *cis*-regulatory elements with more precise, cell type-specific promoters is helping to improve targeting specificity and reduce the risk of retinal toxicity.

A more personalized approach to patient selection is also being emphasized to address variability in therapeutic outcomes, to account for factors such as disease heterogeneity, patient age, and comorbid conditions.[222] Developing tailored clinical protocols will also be crucial for achieving consistent and effective results. ML holds immense potential in the prediction of the risk-benefit ratio for patients undergoing gene therapies. By leveraging large datasets, ML algorithms can analyze patterns that are not be apparent to clinicians or researchers, enabling more precise predictions based on individual characteristics such as genetic mutations, the degree of retinal degeneration, and overall health status. Ensuring long-term safety and efficacy remains a priority, with ongoing research focusing on developing next-generation vectors and therapies that provide durable benefits while minimizing risks.

CONCLUSION AND FUTURE DEVELOPMENT OF rAAV-BASED OCULAR GENE THERAPY

We provided a comprehensive overview of AAV biology, capsid engineering specifically for ocular delivery, and the current status of clinical trials for ocular gene therapy as well as the ongoing challenges and directions of future development. Although the scope of this review captures only a fraction of the expansive field, it underscores the diverse clinical applications of rAAV for many ocular diseases. Despite initial successes and promising advances, challenges persist in developing genetic medicine for IRDs. Immunogenic responses, inflammations, concerns about retinal toxicity related to rAAV *cis*-regulatory sequences, administration routes, rAAV packaging capacity, targeting specificity, and long-term efficacy are all obstacles to the widespread adoption of rAAV that are being individually and collectively improved and optimized to treat many retinal diseases.

Looking ahead, the future of retinal gene therapy seems to be promising. Ongoing research is being done to study the side effects of treatments, exploring selective and transient immunosuppressive regimens in eyes, resolving pre-existing or induced NAbs, refining vector design, developing novel capsids with enhanced transduction and specificity, and alternative delivery routes, which hold the potential to overcome many current limitations. Moreover, highly anticipated results from phase 3 clinical trials of rAAV-based ocular gene therapy have the potential to reinforce confidence in its application and possibly usher in a new era for treating ocular diseases. As the

field advances, interdisciplinary collaborations, continuous monitoring of long-term safety and efficacy, a growing understanding of rAAV biology and disease mechanisms, and engagement between researchers and regulatory agencies will be crucial for the successful clinical translation of rAAV-based ocular gene therapies into mainstream clinical practice.

ACKNOWLEDGMENTS

The authors are grateful to Claudio Punzo for his review and discussion on the manuscript. J-H.W. is supported by the Graduate Education Fund of the American Australian Association. G.G. is supported by NIH grants (R01NS076991-01, P01HL131471-05, R01AI121135, UG3HL147367-01, R01HL097088, R01HL152723-02, U19AI149646-01, and UH3HL147367-04). Figures were created with Biorender.com.

AUTHOR CONTRIBUTIONS

Conceptualization: J-H.W. and G.G. Methodology: J-H.W. and G.G. Formal analysis: J-H.W. Resources: G.G. Data curation: J-H.W. Writing – original draft: J-H.W. Writing – review & editing: J-H.W., W.Z., T.L.G., and G.G. Visualization: J-H.W. Supervision: G.G. Project administration: J-H.W. and G.G. Funding acquisition: G.G. All authors have read and approved the article.

DECLARATION OF INTERESTS

G.G. is a scientific co-founder of Voyager Therapeutics, Adrenas Therapeutics, and Aspa Therapeutics, and holds equity in these companies. G.G. is an inventor on patents with potential royalties licensed to Voyager Therapeutics, Aspa Therapeutics, and other biopharmaceutical companies.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ymthe.2024.10.017.

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