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REVIEW

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Ocular Gene Therapy: An Overview of Viral Vectors, Immune Responses, and Future Directions

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Ocular gene therapy has rapidly advanced from proof-of-concept studies to clinical trials by exploiting the unique advantages of the eye, including its easy accessibility, relative immune privilege, and the ability to use the contralateral eye as a control. An important step forward was achieved with the Food and Drug Administration (FDA) approval of voretigene neparvovec (Luxturna) for the treatment of biallelic RPE65-mutation-associated retinal dystrophies in 2017. Gene therapy is a promising field aimed at treating various inherited and acquired eye diseases. Viral vectors such as adeno-associated virus (AAV) are mainly used to efficiently deliver genes. Despite the immune-privileged status of the eye, viral vector-based therapies can induce immune responses, potentially leading to gene therapy-associated uveitis. Future directions include developing strategies to reduce immune responses while maintaining therapeutic efficacy, optimizing vector selection, and improving delivery techniques. Continued advances in the field of viral vectors, particularly AAV, are expanding the potential applications of gene therapy to treat a variety of ocular diseases. To fully realize the potential of ocular gene therapy, more research and clinical trials are needed to improve these methods, ensure safe and efficient treatments, and ultimately overcome existing obstacles.

INTRODUCTION

Ocular gene therapy has made rapid progress in recent years, evolving from proof-of-concept studies to clinical trials. Gene therapy can be beneficial for the eye because of its well-documented anatomy, relative immune privilege, ease of access and examination, and capacity to use the contralateral eye as a control [1]. A milestone in this field was the Food and Drug Administration (FDA) approval of voretigene neparvovec (Luxturna) in December 2017, the first gene therapy product for RPE65-mediated inherited retinal dystrophies, including Leber congenital amaurosis (LCA) [2,3].

With mutations in over 250 genes identified as causative for inherited eye diseases, the potential scope for gene therapy interventions is enormous [4]. As of 2022, a total of 159 clinical trials focused on gene therapy for eye diseases; 96% of these studies targeted retinal and optic nerve diseases, 2% targeted glaucoma, and the remaining 2% targeted corneal diseases and uveitis [5].

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Abbreviations: FDA, Food and Drug Administration; AAV, Adeno-Associated Virus; LCA, Leber Congenital Amaurosis; GTAU, Gene Therapy-Associated Uveitis; rAAV, Recombinant AAV; ITRs, inverted terminal repeats; NAbs, Neutralizing Antibodies; IRDs, Inherited Retinal Diseases; RPE, Retinal-Pigment Epithelium; DR, Diabetic Retinopathy; DME, Diabetic Macular Edema; AMD, Age-Related Macular Degeneration; RP, Retinitis Pigmentosa; XLRS, X-Linked Retinoschisis; LHON, Leber Hereditary Optic Neuropathy; IOP, Intraocular pressure; RGCs, Retinal Ganglion Cells; NHP, Non-Human Primates; ILM, internal limiting membrane; IVT, Intravitreal; SR, Subretinal.

Keywords: ocular gene therapy, viral vectors, ocular applications, delivery routes, immune responses

Gene therapy offers a promising approach to treat both inherited and acquired diseases by delivering therapeutic genes into target cells [6]. This can be achieved through viral or non-viral vectors. Viral vectors exploit the natural ability of viruses to integrate into human genomes by replacing pathogenic sequences with therapeutic ones, facilitating gene incorporation into target cells [7]. Conversely, non-viral methods use various chemical and physical techniques for gene delivery [8,9]. While initially focused on inherited diseases characterized by loss-of-function mutations, gene therapy now also targets acquired diseases, enabling cells to produce therapeutic drugs *in vivo* [10]. Clinical applications for viral vectors have shown promise. Adenovirus, lentivirus, retrovirus, and adeno-associated virus (AAV) are among the viral vectors that have been examined. The most widely used vector for ocular gene therapy trials is AAV [5,11,12].

However, the journey towards effective ocular gene therapy is not without challenges. Concerns such as immune responses, insertional oncogenesis, and the unpredictable longevity of treatment effects underscore the need for continued research and vigilance in this field [13-15]. Optimizing delivery methods to reduce iatrogenic risks and enhance targeting remains a critical area of study. The success of gene therapy relies on selecting the appropriate delivery system, specific promoter elements, and administration route [16].

TYPES OF VIRAL VECTORS

In gene therapy, three primary vectors are utilized: adenovirus, lentivirus, and AAV, each with unique properties and applications. These vectors differ in their genetic material, delivery mechanisms, and suitability for various diseases.

Adenovirus

Adenovirus is a double-stranded DNA virus known for its ability to efficiently transduce both dividing and non-dividing cells. This ability enables it to insert numerous copies of a gene into a cell, resulting in high levels of protein production [12]. Adenovirus vectors offer practical advantages such as broad tropism, non-integration into the host genome, and a large packaging capacity of approximately 35 kb [17]. Despite being the first vector evaluated in clinical trials, its use has declined. As of 2022, Adenovirus vectors are used in about 1% of clinical trials for ocular gene therapy [5]. The primary reason for this decline is the robust immune response it triggers, leading to inflammation and the elimination of transduced cells [7,17].

Adeno-Associated Virus (AAV)

AAV is the leading vector in ocular gene therapy due to its ability to safely infect various tissues and mediate long-term gene expression [12,18]. AAV is the vector of choice for 87% of ocular gene therapy trials according to a recent study [5]. AAV is a small (25 nm), replication-defective, single-stranded DNA virus from the Parvovirus family [19]. Thirteen different AAV serotypes have been identified in primates [12]. Unlike adenoviruses, AAVs elicit mild immune responses, allowing stable long-term transgene expression, making them suitable for chronic ocular diseases [1,11,20]. Optimized for increased specificity, recombinant AAV (rAAV) has been engineered as a therapeutic agent for a number of ailments [21]. The first rAAV was developed in the early 1980s [22,23]; the first ocular rAAV clinical trial for RPE-associated LCA started in 2007 [24]. rAAV DNA carrier systems have proven highly effective due to several advantages: they enable rapid transgene expression [25,26] and offer long-term treatment efficacy [27], in addition, the capsid composition can be customized [28]. However, rAAVs also have limitations, such as a small gene cassette capacity (up to 4.6 kb plus inverted terminal repeats (ITRs)), the need for high viral loads, and potential humoral immune reactions, including neutralizing antibodies (NAbs) and T-cell responses [11]. With the growing popularity of rAAV as a treatment option, the rising demand has led to difficulties in the current manufacturing process [21].

Lentivirus

Lentiviruses belong to the Retrovirus family, characterized by a single-stranded RNA genome (8-10 kb) [29]. There are five lentiviral serogroups based on their vertebrate hosts, all engineered into recombinant vectors similarly [30]. Both dividing and non-dividing cells can undergo stable transduction by lentiviral vectors [31]. Their genome can be deleted to lessen the inflammatory response, and they can be produced in large quantities [30,32]. Lentiviruses have a high transgene carrying capacity, making them ideal for delivering large therapeutic genes, particularly useful for diseases like Stargardt disease and Usher syndrome [33]. However, concerns include the risk of producing replication-competent lentiviruses, post-transcriptional recombination, and insertional mutagenesis [12,30].

DELIVERY ROUTES

Different ocular delivery modes vary in their invasiveness, targeting precision, and suitability for treating specific conditions (Table 1).

Gene therapy at the anterior segment is accessible through topical, subconjunctival, intrastromal, or intraca-

Table 1. Comparison of Delivery Methods in Ocular Gene Therapy

Delivery Route	Target Tissue	Invasiveness	Advantages	Disadvantages	Clinical Applications
Topical [34,35]	Anterior Segment	Low	•Simple •Safe •Non-invasive	•Limited transduction due to corneal and conjunctival barriers	Anterior segment diseases, Glaucoma
Intravitreal [39-42]	Inner Retina, mainly RGCs	Low	•Less invasive •Broad retinal area coverage	•Immune response risk •Limited penetration to outer retina due to ILM •Systemic biodistribution	IRDs, Glaucoma, Optic Nerve Disorders
Subretinal [39,40,46,47]	RPE, Photoreceptors	High	•Targeted delivery •Immune-privileged space	•Requires surgery •Limited coverage area	IRDs
Suprachoroidal [40,48-50]	Outer Retina	Low/ Medium	•Less invasive •Broad posterior coverage	•Requires multiple layers penetration •Immune response risk •Requires further research	IRDs

IRDs: inherited retinal diseases, ILM: internal limiting membrane, RGCs: retinal ganglion cells, RPE: retinal pigment epithelium.

meral injections. Topical application, though noninvasive and simple, often struggles with effective transduction due to corneal and conjunctival barriers [34,35]. Nanocarriers have been studied and are thought to improve the bioavailability of topically applied drugs [36]. Subconjunctival injection offers a straightforward and safe method for delivering genes to the anterior segment [37]. Some studies have shown that it can also affect posterior segment tissues [38]. Intrastromal injections show promise in targeting the corneal stroma or the entire cornea across different species [19], while intracameral injections effectively transduce anterior segment tissues like the corneal endothelium and trabecular meshwork [19].

Intravitreal, subretinal, and suprachoroidal injections are among the most used gene delivery routed for vitreoretinal disorders. Intravitreal injections are less invasive and easier to perform, can deliver transgenes across a wide retinal area, mainly retina ganglion cells, but are associated with a substantial immune response and may face barriers reaching the outer retina through the internal limiting membrane (ILM) [39-42]. Novel AAV variants have shown the ability to penetrate retina and transduce outer retina cells [43]. Subretinal injections, ideal for retinal-pigment epithelium (RPE) cells and photoreceptors, ensure high transduction efficiency with minimal immune response [40,41,44,45]. It is best suited for inherited retinal diseases (IRDs) and other conditions where retinal structures remain intact [39]. However, vectors are limited to the injected subretinal space, and the technique also requires specialized surgical expertise [46,47]. Suprachoroidal administration with drug delivery via catheters, needles, or microneedles provides an alternative with broad posterior segment coverage but face challenges in delivering the vector through multiple retinal layers [40,48,49].

OCULAR APPLICATIONS IN GENE THERAPY

Viral vectors are emerging as powerful tools for the treatment of various ocular diseases, particularly retinal disorders. In certain cases, the objective is to attain a genetic "cure" by substituting a defective gene product, a strategy frequently used for monogenic IRDs. In other instances, the focus is on transducing host cells to generate a therapeutic protein, effectively creating an ocular protein biofactory. Gene therapy holds significant potential for treating a variety of ocular diseases, including IRDs, corneal diseases, glaucoma, and optic nerve disorders (Figure 1).

Retinal & Optic Nerve Diseases

Viral vector-mediated gene therapy is at the forefront

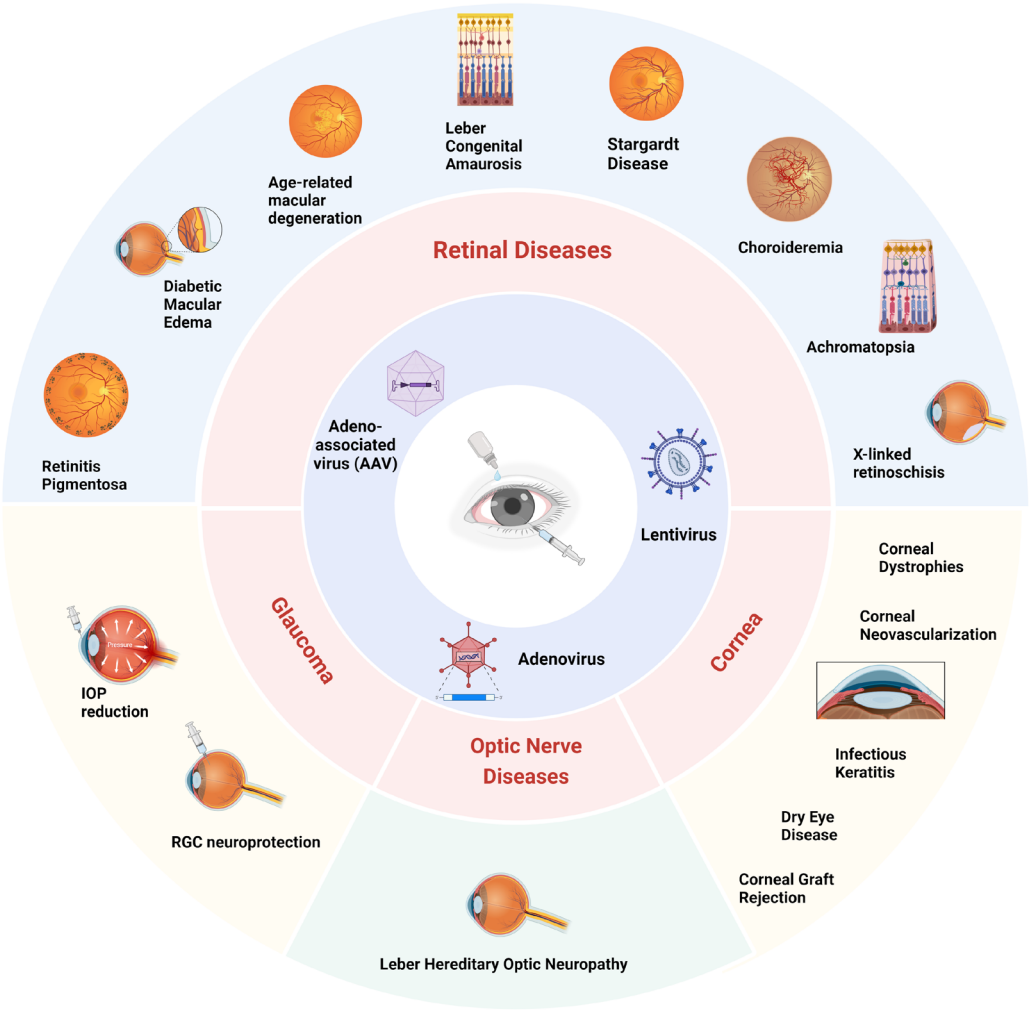

Figure 1. **This figure illustrates the comprehensive landscape of ocular gene therapy using viral vectors**. The inner circle represents the three primary types of viral vectors used: Adenovirus, Adeno-Associated Virus (AAV), and Lentivirus. The second circle categorizes the main ocular applications: Retinal Disorders, Corneal Disorders, Glaucoma, and Optic Nerve Disorders. The outermost circle details specific diseases targeted by gene therapy within each category. Created with BioRender.com.

of innovative treatments for a wide array of debilitating retinal diseases. About 96% of the current clinical trials target retina and optic nerve disorders [5]. IRDs are a diverse group of diseases that cause photoreceptor cell dysfunction and death, leading to severe visual impairment in about one in 4000 people [51]. These disorders, which can be inherited in autosomal dominant, autosomal recessive, X-linked patterns, or associated with mitochondrial DNA mutations, present a significant challenge due to their genetic heterogeneity—over 250 different causative genes have been identified [4,52]. Gene therapy targets both inherited and acquired diseases, such as Diabetic Retinopathy (DR), Diabetic Macular Edema (DME), Age-Related Macular Degeneration (AMD), LCA, Achromatopsia, Retinitis Pigmentosa (RP), Usher Syndrome, Choroideremia, Stargardt Disease, X-Linked Retinoschisis (XLRS), Bietti Crystalline Dystrophy, and Leber Hereditary Optic Neuropathy (LHON) [53-56]. These conditions share the commonality of causing progressive vision loss due to degenerative changes to the photoreceptors, RPE, or the choroid [57]. Using viral vectors, such as AAV, therapeutic genes can be precisely delivered into retinal cells to partially restore the function of photoreceptors, thereby recovering some of the lost retinal functions. The FDA approval of voretigene neparvovec (Luxturna) in December 2017, the first gene therapy product for RPE-65 associated LCA, was a milestone in this field [2,3].

Glaucoma

Glaucoma is a progressive optic neuropathy and is the leading cause of irreversible blindness worldwide. Gene therapy research for glaucoma focuses on two main aspects, reduction of intraocular pressure (IOP) and neuroprotection of retinal ganglion cells (RGCs) [58,59]. Luna et al. evaluated the effects of microRNA 146a (miR-146a) on trabecular meshwork cells and IOP in animal models via viral delivery to the anterior chamber, finding that miR-146a reduced IOP without causing inflammation or visual impairment, indicating potential for glaucoma gene therapy [60]. O'Callaghan et al. demonstrated that an intracameral injection of an AAV-2/9-mediated Matrix Metalloproteinase-3 (MMP-3) gene delivery in mice significantly increased aqueous MMP-3 levels [61]. MMP-3 is an enzyme from the matrix metalloproteinase family that degrades extracellular matrix components in the trabecular meshwork, thus enhancing outflow facility and effectively lowering IOP. This shows potential for therapeutic use in glaucoma, especially in cases resistant to conventional treatments [61]. Chern et al. evaluated a rAAV vector-based gene therapy that induces the biosynthesis of prostaglandin F2α (PGF2α) in the anterior chamber. PGF2α is known to reduce IOP by enhancing aqueous humor outflow through the uveoscleral pathway. Their study demonstrated a sustained pressure reduction in rats over 12 months with reversible control, indicating potential for long-term management of glaucoma [62].

Wang et al. identified and utilized the mouse c-synuclein (mSncg) promoter as an RGC-specific promoter for specific and potent transgene expression in RGCs, demonstrating effective neuroprotection through AAV-mediated CRISPR/Cas9 gene editing against optic neuropathies, such as glaucoma, inflammation, trauma, and genetic deficits [63]. This specificity is crucial for targeted gene therapy providing neuroprotection for injured RGCs and optic nerve, minimizing off-target effects and maximizing therapeutic efficacy [63]. Clinical trials have also investigated the use of small interfering RNA (siRNA) to suppress the synthesis of β2-adrenergic receptors, which are located in the ciliary body and play a critical role in aqueous humor production. By inhibiting these receptors, the siRNA therapy, administered via topical drops, potentially reduces IOP, offering a novel approach for treating individuals with glaucoma (NCT01227291, NCT00990743, NCT01739244, NCT02250612) [64,65].

Corneal Diseases

In the anterior segment, particularly the cornea, gene therapy is promising due to the cornea's accessibility and immune privilege. The route of administration is crucial for the targeted efficacy and safety of gene therapy. The cornea's accessibility allows various methods, like topical application, intrastromal, subconjunctival, and intracameral injections [19]. Viral vectors have been used successfully in preclinical studies to address conditions like corneal fibrosis, scarring, epithelial wound healing, corneal graft survival, neovascularization, corneal dystrophies, and infectious keratitis [19,34,66].

IMMUNE RESPONSES

The eye, as a vital sensory organ, possesses remarkable immunological characteristics that distinguish it as an immune-privileged site. Immune privilege in the eye is crucial for maintaining ocular homeostasis and preserving visual function [67,68]. This privilege is underscored by unique anatomical and physiological features, such as blood-tissue barriers formed by tight junctions at the RPE cell layer, inner-retinal capillaries, and the avascular cornea [68]. These barriers effectively isolate the ocular tissues from systemic immune surveillance, minimizing the risk of inflammation-induced damage and maintaining tissue integrity [67].

Despite the immune privilege of the eye, immune responses can still occur following ocular gene therapy with viral vectors [69]. Factors such as the immunogenicity of the viral vectors, vector dose, delivery route, and the presence of preexisting NAbs can influence the nature and magnitude of immune responses [70]. These factors, along with the complex interplay of individual immune systems, can lead to immune reactions ranging from mild inflammation to more severe responses.

Innate and Adaptive Immune Responses

Ocular gene therapy with viral vectors elicits a complex interplay of innate and adaptive immune responses, which can significantly influence treatment outcomes [69]. Viral vectors have the capacity to trigger an innate immune reaction through various pathways, including the detection of pathogen-associated molecular patterns present on the vector particles or within the vector genome [71]. Innate immune responses are typically the first line of defense triggered by the introduction of viral vectors into the eye. These responses are often dose-dependent, with ocular immune reactions being induced when vector doses exceed a critical threshold as demonstrated by Bainbridge et al. [72], Reichel et al. [20], and Bucher et al. [73].

While innate immunity acts as an immediate, nonspecific initial defense mechanism, adaptive immunity requires several days to form and is highly tailored to a specific pathogen, leading to the establishment of immunological memory [74]. It involves the activation of T and B lymphocytes and the production of antigen-specific antibodies [74]. This enables the organism to generate a quicker and more effective immune response upon en-

countering the antigen for subsequent occasions [20,45].

Humoral Immune Responses

Humoral responses, particularly the development of NAbs, play a crucial role in immune reactions following ocular gene therapy with viral vectors. Preexisting NAbs represent a significant challenge, affecting a substantial percentage of the population. Humans are naturally exposed to environmental AAV strains, leading to host preexisting anti-AAV NAbs. It is estimated that up to 95% of the general population has been infected by wild-type AAVs, and about 50% of the population carries NAbs [75]. Boutin et al. have shown varying prevalence of anti-AAV antibodies with anti-AAV1 and anti-AAV2 total IgG levels being the highest among the other types. Cross-reactions are also significant, with the lowest neutralizing factor seroprevalences observed for AAV8 and AAV5. Therefore, they suggested that vectors based on AAV5, AAV8, and AAV9 might thus have an advantage for gene therapy in humans [76].

Exposure of viral vectors to NAbs in the vitreous can induce inflammation and decrease vector integrity [42,77]. Intravitreal AAV injections elicit stronger anti-capsid and neutralizing antibody responses in serum and ocular fluids compared to subretinal injections [20]. Cukras et al. demonstrated an increase in systemic AAV8 antibodies after vector application, with a dose- and time-dependent increase in serum AAV8 neutralizing antibody titers [78]. Pre-existing NAbs may affect transgene expression after intravitreal injection of AAV and potentially reduce the therapeutic efficacy of second eye treatment [77]. However, it is difficult to determine which concentrations of NAbs are clinically relevant and influence therapeutic outcomes [79].

Understanding the impact of humoral responses and preexisting NAbs on ocular gene therapy is crucial for optimizing treatment strategies and improving patient outcomes.

Gene Therapy-Associated Uveitis

Despite the success of viral-mediated retinal gene therapy and the immune-privileged status of the eye, there is increasing evidence that gene therapy can induce both local and systemic immune responses, leading to ocular inflammation [70]. Gene Therapy-Associated Uveitis (GTAU) is an inflammatory condition affecting the uveal tract of the eye following the administration of gene therapy using viral vectors. GTAU has been reported in various studies despite the use of systemic anti-inflammatory treatments such as corticosteroids [80,81]. Clinical manifestations of GTAU can vary widely, including anterior chamber cells and flare, vitritis [78,82,83], retinitis [84,85], endophthalmitis [81] and choroidal scarring or atrophy [86]. These symptoms reflect the extensive intraocular inflammation that can occur.

Several risk factors have been identified that increase the likelihood and severity of GTAU, including vector dosage, route of delivery, and vector contaminants. Xiong et al. found that a dose of 10^{11} vector genomes (vg) per eye led to toxicity in their gene therapy study, suggesting that the higher dose might be the reason for the observed toxicity of the AAV-Rho-GFP vector [87]. Timmers et al. found that intravitreal dosing of AAV viral vectors transiently increased cellular inflammation in the aqueous humor and induced more sustained inflammation in the vitreous. They observed that reducing the total capsid dose by removing empty AAV capsids reduced inflammation and improved viral transduction [42]. Intravitreal route is also considered more immunogenic than subretinal space [78]. Potential contaminants in the vector preparation could also trigger immune responses [70]. Bucher et al. concluded that AAV preparations can contain immunogenic extra-viral DNA components which can trigger lot-specific inflammatory immune responses [73]. Clinical grade AAV preparations are routinely checked for these contaminants, but there are no uniformly agreed specifications as to what levels are acceptable [70].

Management of GTAU typically involves corticosteroids to control inflammation, with both systemic and local corticosteroids being used to treat acute inflammatory signs [80,81]. Prophylactic steroid treatment is often included in clinical trial protocols to prevent inflammation [70], but alternative immune modulation methods are also being explored due to the risks associated with prolonged corticosteroid use [88,89].

GTAU remains a significant concern in ocular gene therapy, emphasizing the need for ongoing research to better understand its pathophysiology and develop improved treatment strategies.

Determinants of Immune Responses

In ocular gene therapy, understanding the determinants of immune responses is crucial for optimizing treatment efficacy and minimizing adverse effects. Each factor influences the nature and magnitude of the immune response, impacting the overall success of the therapy. By comprehensively analyzing these determinants, researchers and clinicians can develop tailored strategies to enhance the safety and effectiveness of gene therapies for various ocular conditions.

Vector Selection: Vector selection plays a crucial role in determining immune responses following ocular gene therapy. Adenovirus vectors were initially appealing for gene therapy due to their large packaging capacity, efficient transduction of many non-dividing cell types, and ease of production [7]. However, Adenovirus vectors trigger a strong innate immune response through com-

Table 2. Determinants of Immune Responses in Ocular Gene Therapy

Determinant	Description	Impact on Immune Response	Mitigation Strategies
Viral Vector Type [7,17,30,69,74]	Different vectors (AAV, adenovirus, lentivirus) with varying immunogenicity.	Determines the type and strength of the immune response.	Use less immunogenic vectors (AAVs are less immunogenic)
Vector Dose [78,80,91,93,94]	Amount of viral vector administered.	Higher doses can increase immune and inflammatory responses.	Optimize dosing strategies.
Delivery Route [41,42,45,50,78]	Method of administration (subretinal, intravitreal, suprachoroidal).	Affects the extent and localization of immune responses.	IVT route more immunogenic. SR route achieves compartmentalization.
Preexisting NAbs [42,74-79]	NAbs present before therapy due to prior exposure to wild-type viruses.	Can neutralize the vector, reducing efficacy and increasing inflammation.	Screen for NAbs, use less common AAV serotypes.
Vector Modification [73,75,95-98]	Alterations to the vector to reduce immunogenicity (eg, capsid modifications, DNase treatment).	Can reduce immune recognition and enhance therapeutic efficacy.	Modify capsids to reduce immunogenicity.
Contaminants in Vector Preparation [73,99]	Presence of extra-viral DNA or other impurities in the vector preparation.	Can trigger unwanted immune responses.	Ensure high purity in vector preparations.
Immunosuppressive Treatments [70,80,81,83,84,88,89]	Use of corticosteroids or other immune-modulating drugs before or after therapy.	Helps manage and mitigate immune responses and inflammation.	Prophylactic or therapeutic immunosuppressive treatments. Requires more research.
Host Immune Status, Genetic, Demographic Factors [45,70,73,74,79,100]	Medical history of the patient, overall immune health, genetic variations, previous infections, demographic factors.	Influences individual variability in immune response.	Patient history, genetic screening, personalized medicine.

AAV: Adeno-associated virus, NAbs: Neutralizing Antibodies, IVT: Intravitreal, SR: Subretinal

plement activation and both Toll-like receptor (TLR)-dependent and TLR-independent pathways [17,69]. Lentivirus vectors, while integrating into the host genome and potentially offering long-term expression, also pose immunogenicity risks [30]. In contrast, AAV vectors are less immunogenic. Currently, they are the leading viral vectors for ocular gene therapy. Nonetheless, AAVs are still capable of eliciting immune responses, including GTAU and the development of NAbs against the vector capsid, which can impair transduction efficiency [74,90]. Different serotypes exhibit different transduction efficiency and immunogenicity in the ocular microenvironment [50,74]. Since AAVs are non-pathogenic, using them as vectors initially appears to be a better choice to prevent an immune response. However, the widespread pre-existing immunity in a significant portion of the population poses a challenge, as many capsids have been found to be neutralized, even in the eye. Choosing the appropriate viral vector involves balancing these immune response considerations to optimize therapeutic efficacy and minimize adverse effects.

Vector Dose: Preclinical and clinical studies have demonstrated that immune responses are significantly influenced by the vector dose. In non-human primates (NHP), high doses delivered via subretinal or intravitreal injections have been linked to various immune responses. These include innate retinal responses, perivascular and inner retinal cell infiltration, inflammation, loss of RPE, chronic choroidal inflammation, retinal thinning, and infiltration of aqueous and vitreous cells [45,81,91,92]. Beltran et al. indicated that clinical and histopathological signs of inflammation were most severe when titers of 10^{12} - 10^{13} vg (viral genomes)/ml were used [93]. Ramachandran et al. reported that subclinical signs of inflammation (elevated GFP expression and retinal infiltrates) after injection with the highest dose (10^{12} vg) of either serotype [91]. Clinical studies also indicate that ocular immune responses are generally triggered in higher vector dosage. Cukras et al. reported a dose-related ocular inflammation after injection with three different doses of 10^{9} vg/eye, 10^{10} vg/eye, and 10^{11} vg/eye [78]. Bainbridge et al. reported intraocular inflammation in patients receiving higher doses (10^{12} vg/eye vs 10^{11} vg/eye) [80]. Additionally, AAV administration can lead to a dose-dependent increase in NAbs [94]. The variability in responses may be influenced by numerous factors, including the specifics of the vector delivery, the underlying disease, and the use of immunosuppressive treatments before and after vector administration.

Delivery Route: The route of administration significantly affects the severity of immune and inflammatory reactions in ocular gene therapy. While intravitreal application of AAV leads to considerable systemic biodistribution, subretinal injection achieves effective compartmentalization and can temporarily shield the injection site from immunogenic scrutiny, potentially offering advantages [41,42]. Reichel et al. compared intravitreal and subretinal delivery in NHP eyes, concluding that intravitreal delivery prompted a significant humoral immune response, suggesting that subretinal delivery may be preferable to intravitreal application from an immunological standpoint [45].

Another promising approach is suprachoroidal delivery, which is less invasive than the subretinal route and can result in lower immune responses than intravitreal route. This method allows vectors to reach the outer retina effectively. Studies suggest that suprachoroidal space may have better retention of viral particles than the vitreous cavity [50]. Additionally, the absence of immune privilege in the suprachoroidal space highlights the need to carefully address the impact of preexisting NAbs on suprachoroidal gene therapy [40].

TREATMENT STRATEGIES

Despite the success of AAV-based gene therapies, there is increasing evidence that AAV vectors can be immunogenic, leading to both local and systemic immune responses, including ocular inflammation, potentially limiting the therapeutic efficacy of the treatment. To address these challenges, several strategies have been developed to suppress AAV-induced immune responses and inflammation.

First, reducing the vector dose is crucial. Clinical trials have shown that inflammation occurs predominantly in high-dose cohorts, and lower doses can mitigate this risk [78,80,91]. A dose of 1 to 1.5 × 10^{11} vg/eye is considered both effective and safe, and associated with minimal inflammation [5]. Some strategies focus on modifying the AAV vector to reduce its immunogenicity. One approach involves developing AAV capsids resistant to NAbs, such as Tse et al. who generated synthetic AAV variants that evade neutralizing sera from different species [95]. Mevel et al. successfully developed chemically modified capsids that also showed reduced interactions with neutralizing antibodies [96]. Bucher et al. reported another promising method is to treat AAV vectors with DNase to reduce innate immune responses by removing extra-viral DNA impurities without affecting the transduction efficiency [73]. In a recent study, Mevel et al. described the development of a mannose-coupled AAV, a second generation AAV vector which showed better therapeutic outcomes [97] (Table 2).

Immunosuppressive strategies primarily involve the use of corticosteroids. Most inflammatory responses reported in clinical trials are temporary and resolve with time or further corticosteroid treatment [83,84,101]. Transient immunosuppression with corticosteroids is

widely used in retinal gene therapy. Management of GTAU typically involves corticosteroids to control inflammation, with both systemic and local corticosteroids being used to treat acute inflammatory signs [80,81]. Prophylactic steroid treatment is often included in clinical trial protocols to prevent inflammation [70], but alternative immune modulation methods are also being explored due to the risks associated with prolonged corticosteroid use [88,89].

Furthermore, implementing stringent screening and exclusion criteria in clinical trials is crucial to minimizing immunogenicity. For instance, excluding patients with pre-existing eye conditions, those dependent on immunosuppressive medications, or those with high levels of NAbs against AAV can help reduce the risk of adverse immune responses.

FUTURE DIRECTIONS

The future of viral vectors in ocular gene therapy holds significant promise and ongoing challenges. Viral vectors, particularly AAVs, have emerged as powerful tools for delivering therapeutic genes to target cells in the eye, offering advantages such as efficient transduction and long-term gene expression. However, several areas warrant further exploration and refinement.

Firstly, enhancing vector specificity and efficiency is crucial. While AAVs have shown remarkable safety profiles, improving their tropism for specific cell types within the retina could enhance therapeutic outcomes and reduce off-target effects. This may involve the development of novel capsid variants through techniques like directed evolution or rational design, allowing for precise targeting of desired cell populations [40]. Secondly, addressing immune responses remains a key challenge. Preexisting NAbs against viral vectors can hinder transduction efficiency and increase the risk of immune reactions, particularly with repeated administrations [74]. Strategies to mitigate immune responses, such as immune modulation or the use of alternate serotypes with lower immunogenicity, will be essential for optimizing the safety and efficacy of viral vector-based therapies.

Furthermore, advancements in vector delivery methods are anticipated. While subretinal and intravitreal injections are commonly used, novel approaches such as suprachoroidal delivery with microcatheters or non-invasive techniques like topical application or gene editing technologies may offer advantages in terms of safety, ease of administration, and patient comfort [34,40]. A recent clinical trial compared the use of robot assist devices against vitreoretinal surgeons not using assistive devices [102]. The first in-human study utilizing a robotic device to assist subretinal drug delivery in patients undergoing vitreoretinal surgery represents a significant milestone in ophthalmic surgery and potential treatment modalities [102]. Even though the difference in outcomes was not statistically significant between the two groups, the fact that both groups were able to successfully complete the procedure suggests that these devices have potential utility [102]. This technology could hold particular promise for gene therapy applications, where precise and targeted delivery of therapeutic agents to specific retinal layers is essential. By overcoming the challenges associated with subretinal administration, such as variability in injection depth and potential damage to surrounding tissues, robotic assistance could pave the way for more effective and safer gene therapy treatments for various retinal diseases [44].

Proceeding to other applications, optogenetics presents a revolutionary approach for restoring neuronal function in neurodegenerative eye diseases like RP, offering hope for individuals facing blindness due to photoreceptor loss [103-105]. The most widely investigated approach for delivery for optogenetic agents is with gene therapy vectors such as AAV [105]. While IRDs primarily involve photoreceptor degeneration, inner retinal neurons such as bipolar cells and ganglion cells often remain structurally intact even in advanced stages of the disease [106]. Optogenetics presents a promising therapeutic approach by targeting these residual cells, independent of the underlying genetic mutation. In a groundbreaking case, optogenetic therapy involving intraocular viral vector injection combined with light-stimulating goggles led to partial functional recovery in a blind patient, marking a significant milestone in the field [107]. By targeting residual retinal neurons with light-sensitive proteins, independent of the underlying genetic mutation, optogenetics aims to restore visual function [103]. Ongoing research and clinical trials are exploring this approach, with the potential to reverse visual loss in advanced retinal degeneration [103-105].

As AAV stands as a cornerstone in gene therapy, the increased demand has created challenges for the existing manufacturing methods [21]. While chromatographic methods hold promise for enhancing purification efficiency and reducing costs, they typically need to be tailored to each specific AAV serotypes. Florea et al. have recently demonstrated the efficacy of the POROS CaptureSelect AAVX affinity resin in efficiently capturing a diverse range of 15 AAV serotypes, as well as its ability to be regenerated multiple times without compromising its efficiency or leading to contamination [108]. More techniques need to be established to increase the efficiency and decrease the cost of production.

Overall, the future of viral vectors in ocular gene therapy is characterized by continued innovation, with ongoing efforts aimed at refining vector design, delivery methods, and therapeutic strategies to maximize efficacy,

safety, and accessibility for patients with ocular diseases.

CONCLUSION

Ocular gene therapy stands at the forefront of translational medicine, promising to revolutionize the treatment of a wide array of hereditary and acquired ocular diseases. The FDA's approval of Luxturna has set a precedent, demonstrating the potential of gene therapy to achieve substantial and lasting therapeutic effects. The use of viral vectors, particularly AAV, has been instrumental in these advancements, offering efficient gene delivery and sustained gene expression. However, the field faces significant challenges, primarily related to immune responses and the optimization of delivery methods. Addressing these issues requires a multifaceted approach, including the development of less immunogenic viral vectors, precise delivery techniques, and effective immunosuppressive strategies. Continued research and clinical trials are crucial for refining these technologies, ensuring their safety, and maximizing their therapeutic potential. As the field progresses, it is essential to balance innovation with caution, rigorously assessing long-term outcomes and potential risks. The ongoing evolution of ocular gene therapy holds immense promise for patients with currently untreatable conditions, and with sustained effort and collaboration, it is poised to become a cornerstone of ocular disease management.

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