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Gene therapy for age-related macular degeneration: a promising frontier in vision preservation

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Abstract

Age-related macular degeneration (AMD) is a leading cause of central vision loss, progressively impairing the retina and affecting millions worldwide. By 2040, global cases of AMD are projected to reach 300 million, posing a significant public health challenge. While early AMD may only cause mild visual impairment, advanced stages, particularly neovascular (wet) and non-neovascular (dry) AMD, can lead to severe vision loss or legal blindness, substantially affecting daily life. The introduction of anti-angiogenic therapies has revolutionized wet AMD treatment, offering a high probability of preserving or improving vision. However, these therapies do not halt AMD progression, and no definitive treatments exist for dry AMD. The limitations of current therapies, such as frequent injections and treatment resistance, underscore the urgent need for novel strategies. Gene therapy, which has shown success in treating other hereditary retinal diseases, offers a promising long-term solution for AMD by targeting retinal cells to produce therapeutic proteins. This review explores the potential of gene therapy for AMD, examining recent clinical trials and future treatment directions.

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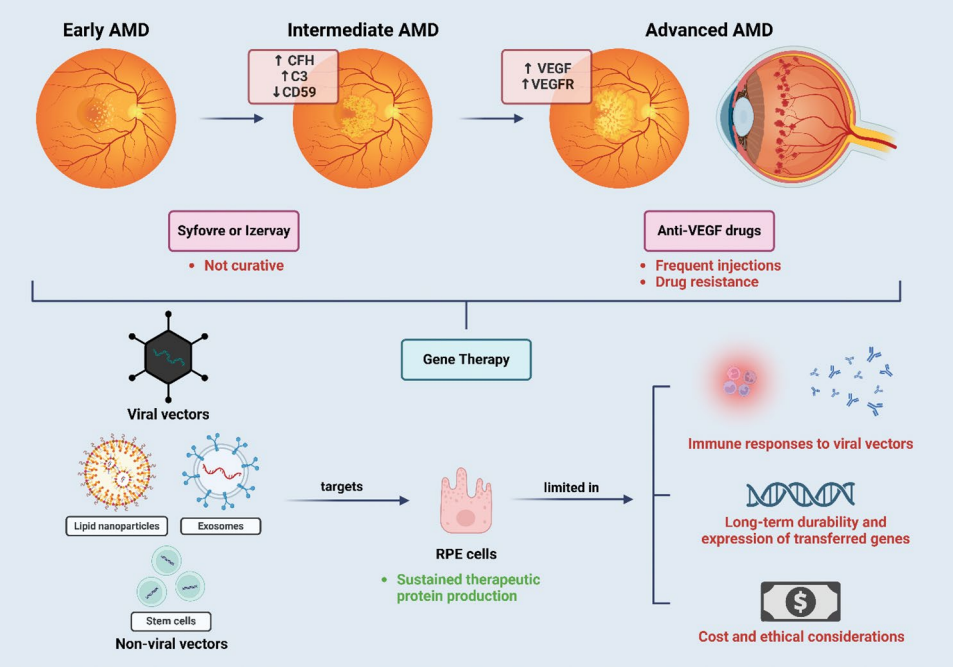
Graphical Abstract



Keywords Macular degeneration, Age-related macular degeneration, Retinal diseases, Genetic therapy, Gene delivery systems

Introduction

Age-related macular degeneration (AMD) is a major cause of vision loss, primarily affecting the central region of the retina and leading to progressive visual impairment. The disease progresses through four distinct stages, from minimal early changes to severe vision impairment, potentially culminating in legal blindness. Early signs include drusen accumulation and alterations in the retinal pigment epithelium (RPE), while advanced stages, particularly neovascular (wet) and non-neovascular (dry) AMD, lead to significant central vision loss, severely impacting daily activities and autonomy. The global prevalence of AMD affected 200 million people in 2020 and is expected to rise to nearly 300 million by 2040, posing a substantial public health challenge with significant social and economic implications [1, 2].

AMD is classified into four progressive stages based on distinct pathological features [3]: stage 1 (normal ageing) is characterised by scant drusen deposits (< 63 μm) and no detectable anomalies in RPE cells; stage 2 (early AMD) includes intermediate-sized drusen (63 μm to 124 μm) without RPE cell irregularities; stage 3 (intermediate AMD) is marked by the significant presence of drusen, including at least one large drusen (> 125 μm), often coupled with pigmentary abnormalities; and stage 4 (advanced AMD), which includes geographic atrophy (GA) involving both the fovea and neovascular maculopathy, both of which are associated with vision loss [4, 5].

The Age-Related Eye Disease Study (AREDS), which simplified the severity scale for AMD categorization and described the degree of vision loss, has been validated by Liew et al. [6] Early AMD is typically associated with minor visual disruptions such as reduced reading ability or the presence of a central scotoma, while intermediate AMD may present with larger drusen or abnormal pigmentation. In advanced AMD, central vision is significantly compromised, leading to pronounced visual disability [7].

Anti-angiogenic pharmaceuticals have revolutionized the management of wet AMD, offering significant improvements in visual outcomes prognoses. Prior to their development, progression to vision loss in wet AMD was almost inevitable [8]. Anti-angiogenic treatments now provide a greater than 90% probability of improving visual acuity by three lines on an eye chart following a two-year treatment period [9]. However, these treatments specifically target wet AMD, focusing on the abnormal blood vessel formation driven by vascular endothelial growth factor (VEGF). Unfortunately, no similar treatments were available for dry AMD, especially its advanced form, GA, which posed a significant challenge for clinicians [10]. While this strategy effectively

manages the immediate pathological features, it offers no preventive or protective benefits against the onset of AMD. In addition, the lack of effective treatments for dry AMD is largely due to the intricate interplay of genetic, environmental, and age-related factors that contribute to the disease [10, 11]. Recent breakthroughs, however, offer new hope. Two newly FDA-approved treatments, pegcetacoplan (Syfovre) and avacincaptad pegol (Izervay), target the complement system, which plays a crucial role in the progression of GA, a late stage of dry AMD. Pegcetacoplan inhibits C3, while avacincaptad pegol blocks C5, both effectively reducing the rate of GA lesion growth in clinical trials [12–15]. These treatments represent the first effective therapies for dry AMD, specifically targeting GA, and mark a significant advancement in the management of this previously untreatable condition. However, deciphering complexities in AMD is essential for identifying more effective therapeutic targets for both wet and dry AMD.

Clinical observations reveal a gradual decline in visual acuity among wet AMD patients undergoing anti-angiogenic therapy. This deterioration is often linked to suboptimal treatment responses, the partial efficacy of the therapy, and the ongoing development of fibrotic scarring or GA [16–20]. These challenges, along with the demanding regimen of frequent injections, highlight the urgent need for more advanced treatment options for AMD.

In recent years, gene therapy has gained significant attention as a promising therapeutic approach for various hereditary retinal disorders, including Leber's congenital amaurosis (LCA), choroideremia, retinitis pigmentosa (RP), Usher syndrome, Stargardt disease, Leber hereditary optic neuropathy, achromatopsia, and X-linked retinoschisis. The success of gene therapy in managing LCA has fueled optimism about its potential application in AMD, a condition with far greater prevalence and societal impact [21]. Notably, a single gene therapy intervention targeting RPE cells in AMD holds promise for the sustained production of anti-angiogenic and other therapeutic proteins, offering the potential for long-term treatment [22].

Given these considerations, this review highlights the critical role of gene therapy in AMD treatment, providing an in-depth analysis of the latest clinical trials and recent advancements in this evolving field (Fig. 1).

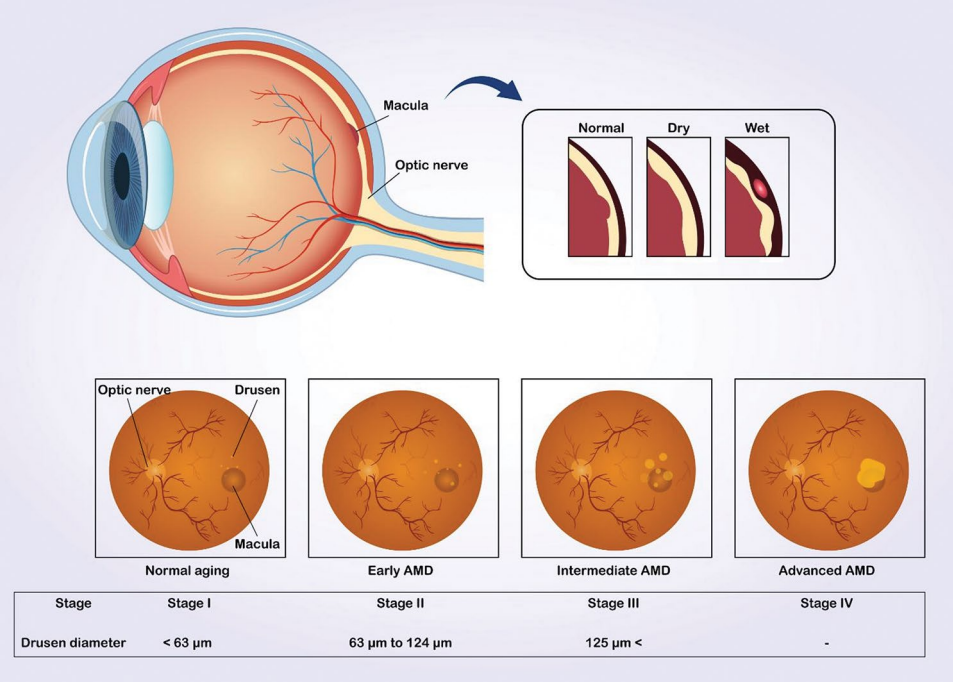


Fig. 1 Pathophysiology of AMD. An overview of age-related macular degeneration (AMD), a leading cause of vision loss in older adults, is provided here to highlight the AMD's anatomical impact, clinical subtypes, and progression through defined stages. The top left schematic shows the anatomy of the eye, emphasizing the macula and optic nerve. Adjacent cross-sectional views differentiate between a healthy retina, dry AMD (characterized by drusen accumulation and retinal thinning), and wet AMD (marked by abnormal blood vessel growth and leakage beneath the macula). There are also four clinical stages of AMD based on drusen size and retinal appearance: Stage I (Normal Aging): Presence of small drusen (< 63 µm), commonly seen with aging but not indicative of AMD; Stage II (Early AMD): Characterized by medium-sized drusen (63–124 µm) with minimal visual symptoms; Stage III (Intermediate AMD): Defined by large drusen (> 125 µm) and/or pigmentary changes, often associated with mild to moderate visual impairment; and Stage IV (Advanced AMD): Includes geographic atrophy (dry AMD) or neovascularization (wet AMD), resulting in significant central vision loss

Genetic basis of AMD and therapeutic targets

Genetic risk factors and their association with environmental factors

Genetic factors play a critical role in the development of AMD, with over 100 genes or loci implicated in the condition. Among these, complement factor H (CFH) and HtrA serine peptidase 1 (HTRA1) are some of the most significant. The CFH gene, located on chromosome 1q32, encodes Complement Factor H, a key regulator of the complement system [23]. The 402 H allele of CFH is a well-established risk factor for AMD [24–26]. This allele hinders the ability of CFH to clear oxidative lipids from the RPE, triggering a cascade of negative reactions. Specifically, it impairs CFH's role in inhibiting the conversion of C3 to C3b and its subsequent degradation [23, 26–28], leading to uncontrolled complement activation, which contributes to AMD onset. In addition to CFH, other complement-related genes influence AMD risk. The genes CFB and C2, which are implicated in the activation of the alternative and classical complement pathways, respectively, have opposing impacts. The CFB R32Q and R32Q/IVS10 haplotypes are linked to reduced AMD risk, while certain mutations in C3, such as R102G, correlate with AMD progression [29, 30]. Furthermore, CFI, which is regulated by CFH to deactivate C3b, has variants that can either be protective or increase AMD risk [31].

A variety of genes involved in lipid metabolism have been identified to contribute to AMD risk [32–34]. A key gene in this context is apolipoprotein E (APOE), which exists in three primary forms: ε2, ε3, and ε4. The ε4 allele is associated with a reduced risk of AMD compared to the ε3 allele [35, 36]. This protective effect may stem from the ε4 variant's ability to expedite the clearance of lipids, cholesterol, and cellular debris from the RPE, thereby preventing the buildup of these substances and the subsequent formation of drusen, an early marker of AMD [36, 37]. In contrast, the ε2 allele may contribute to an increased risk of AMD by potentially promoting the expression of growth factors within RPE cells [38], highlighting the complex relationship between APOE alleles and AMD progression.

Beyond APOE, other genes involved in lipid metabolism, such as TIMP3 and LIPC, have also been linked to a higher risk of developing AMD [33, 39]. Certain genetic variants, such as rs9621532 in TIMP3 and rs10468017 in LIPC, have been pinpointed as potential contributors to AMD susceptibility. Additionally, genes coding for proteins that function in the retina, including CETP, LPL, and ABCA1, are believed to play a role in AMD pathogenesis, possibly through their involvement in drusen formation, although further research is needed to confirm these associations [33, 40].

Genome-wide association studies (GWAS) and other research have identified over 100 loci associated with AMD susceptibility [41]. These loci encompass genes involved in diverse cellular processes. While most AMD-associated genes affect multiple types of the disease, some have more specific associations. For instance, TLR3, which plays a role in innate immunity, is linked exclusively to dry AMD. Conversely, a rare variant within FGD6 has been associated specifically with polypoidal choroidal vasculopathy (PCV), a subtype of wet AMD. These findings underscore the intricate relationship between genetics and the distinct subtypes of AMD [42].

Genetic predisposition, combined with environmental factors, plays a synergistic role in the development of AMD. For example, smoking has been shown to amplify the risk associated with certain AMD-associated genetic variants. The CFH rs1061170 variant, which alters CFH's binding affinity to complement components C3 and C3b, significantly increases susceptibility to AMD in smokers [27, 43, 44]. In a similar vein, the presence of any HTRA1, when coupled with smoking, further elevates the risk of developing AMD [45].

Further research has shed light on additional gene-environment interactions pertinent to AMD. The AREDS highlighted a notable interaction between the CFH 402 H allele and body mass index (BMI) in relation to AMD risk [46]. Similarly, the Blue Mountains Eye Study (BMES) found that a diet rich in fish offers greater protective benefits against late-stage AMD for individuals with the CFH 402Y variant [47]. Moreover, the intake of antioxidant nutrients appears to reduce the risk of early-stage AMD in those with a genetic predisposition due to CFH and HTRA1 variations [48, 49]. Moreover, increased levels of C-reactive protein (CRP) may further exacerbate the risk associated with specific HTRA1 variants [50].

Gene-gene interactions also play a role in increasing AMD risk. Notably, the combined effect of specific genetic variants on chromosomes 6q16.2 and 18q22.1 significantly elevates AMD risk, particularly in individuals who smoke over extended periods [51]. Research by Schmidt et al. suggests that the APOE genotype influences the impact of smoking on AMD risk, especially in the development of choroidal neovascularization (CNV). Their findings indicate that smoking is particularly detrimental to individuals carrying the APOE ε2 allele [52]. Figure 2 underscores that AMD is genetically heterogeneous, with both protective and risk-conferring variants influencing disease onset and progression. Complement pathway genes (e.g., *CFH*, *C3*, *CFB*) play a central role, reflecting the importance of immune dysregulation in AMD pathophysiology. Understanding these genetic associations not only enables risk stratification and early detection but also informs the development of targeted therapies, such as complement inhibitors and gene-based interventions, tailored to individual genetic profiles.

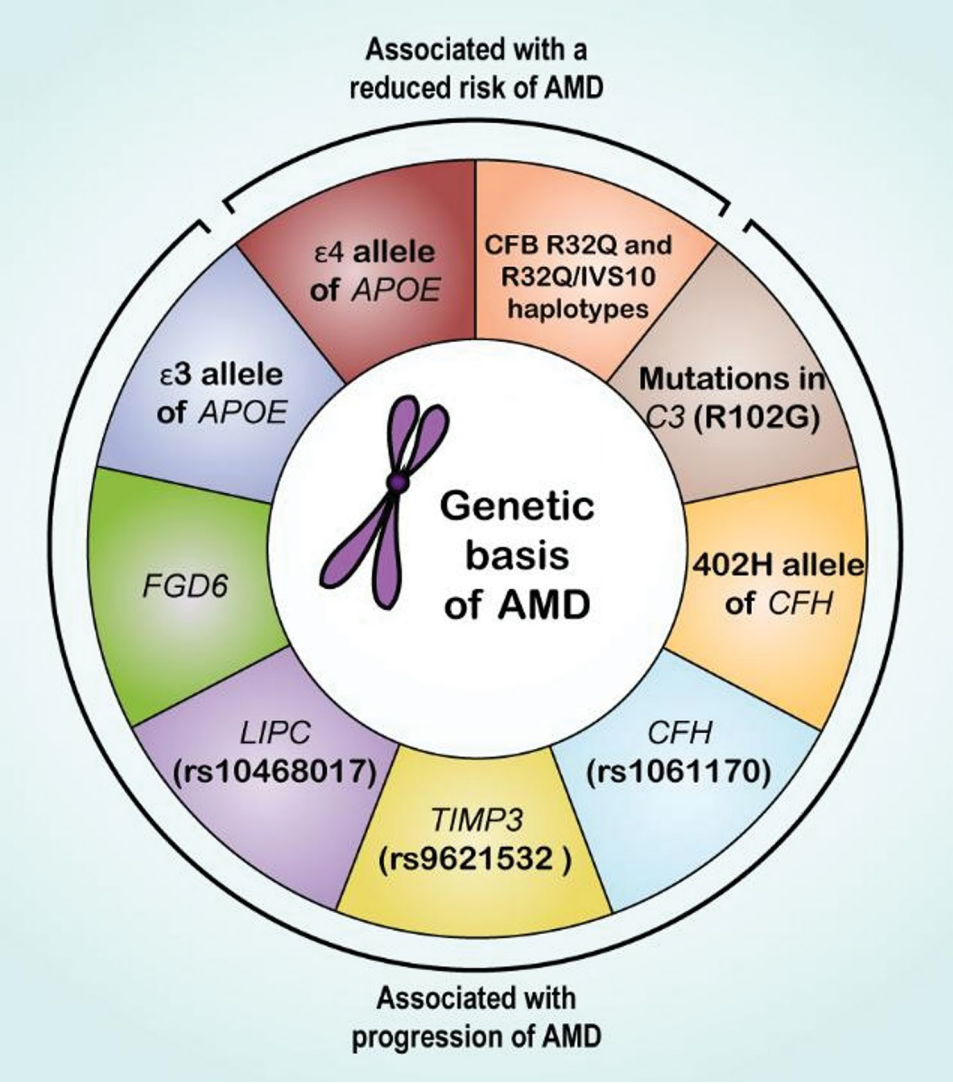


Fig. 2 Genetic basis of age-related macular degeneration. The genetic landscape influencing susceptibility to AMD, distinguishing between variants associated with either reduced risk or progression of the disease is illustrated here. The central concept highlights that AMD is a complex, multifactorial disorder with strong genetic underpinnings, involving both protective and risk-associated alleles across multiple genes implicated in lipid metabolism, inflammation, and extracellular matrix remodeling

Identification of AMD-associated genes: role of lipid metabolism, autophagy, and complement pathway genes

Accumulating data from genetic research, clinical observations, and histological examinations suggest a crucial link between aberrant lipid metabolism and the onset of AMD [53, 54]. Genes involved in cholesterol transport, such as APOE, ABCA1, LIPC, and CETP, have shown a strong correlation with AMD risk. Additionally, elevated plasma levels of high-density lipoprotein (HDL) are associated with a reduced risk of AMD. The buildup of cholesterol-rich deposits beneath the RPE, known as sub-RPE drusen, is a key hallmark of AMD progression [55]. This connection is further supported by animal models of Stargardt disease, best disease, and specific gene deletions, which exhibit excessive cholesterol accumulation in the RPE before any pathological changes occur. This highlights the importance of maintaining tight cholesterol regulation within the RPE [56, 57]. Understanding how lipid metabolism is regulated in the RPE and the mechanisms by which cholesterol disrupts its function is essential for advancing our knowledge of AMD pathogenesis.

Recent studies suggest a mechanism in which bisretinoids, derivatives of the retinal chromophore recycling process, trigger the secondary accumulation of cholesterol and ceramide within the RPE [58]. The distinct cone-like structure of bisretinoids, featuring a diminutive hydrophilic head and an expansive hydrophobic tail, disrupts lysosomal membrane structure. Bisretinoids embed themselves beneath the head groups of adjacent

phospholipids to minimize water exposure. Cholesterol, which shares a similar cone-like shape, competes for the same space as bisretinoids. Experiments using synthetic membranes containing both compounds have shown that bisretinoids can displace cholesterol, causing it to become internalized and sequestered within lysosomes [58]. This excess cholesterol subsequently ensnares bis(monoacyl)glycerophosphate (BMP) within lysosomes. Increased levels of lysosomal cholesterol and BMP have been observed in human RPE cells cultured in vitro and in Abca4 knockout mice. MALDI imaging techniques have also verified heightened BMP levels in the RPE of these mice [59]. BMP acts as a cofactor for acid sphingomyelinase (ASM), an enzyme located in lysosomes that converts sphingomyelin into ceramide. Cholesterol-induced activation of ASM in Abca4 knockout mice leads to a substantial accumulation of ceramide in the RPE. This ceramide buildup may disrupt microtubule stability and membrane dynamics in the RPE, potentially affecting cellular metabolism and mitochondrial function—topics that will be addressed in subsequent sections. [60–62].

In the retina, the signaling lipid ceramide requires precise regulation [63]. Research utilizing animal models indicates that an excess of ceramide impairs various retinal cells, including RPE cells, photoreceptors, and endothelial cells. Such impairments are evident in conditions such as retinitis pigmentosa, light-triggered photoreceptor cell death, and diabetic retinopathy [64, 65]. Additionally, human clinical studies have identified a link between AMD and elevated ceramide levels, both systemically and within the RPE layer. Notably, these heightened ceramide levels are absent in healthy control groups [66–68].

Autophagy, the intracellular degradation system that recycles cellular debris, is vital for the maintenance of RPE cells. Given their non-dividing nature and high metabolic demands, these cells rely heavily on autophagy to manage waste [69]. Mouse models with targeted deletions of autophagy-related genes in RPE cells (such as RB1CC1, ATG5, ATG7) have highlighted autophagy's essential role in preserving RPE integrity and visual function. These models demonstrate a gradual loss of RPE cells, leading to subsequent retinal degeneration [70, 71].

Human tissue studies have revealed reduced autophagosome marker levels (lipidated LC3) and an accumulation of persistent cellular waste (p62/SQSTM1) in the RPE of AMD patients compared to non-AMD controls [72, 73]. Until recently, the reasons for impaired autophagy in aging and AMD-affected RPE were not well understood. Advanced imaging techniques have now shown that elevated cholesterol and ceramide levels, triggered by bisretinoids, lead to the buildup of rigid, acetylated microtubules within RPE cells. These altered microtubules hinder the efficient movement of autophagosomes within the RPE [60]. Furthermore, the acetylation of microtubules disrupts the proper positioning of lysosomes. Normally, these organelles are transported along the plus-ends of microtubules by kinesin motors, which in polarized RPE cells are oriented toward the cell base. However, acetylated microtubules have a higher affinity for kinesin motors, causing lysosomal clustering near the cell nucleus when exposed to bisretinoids [62]. This disruption in autophagosome transport and lysosome positioning results in compromised autophagy and the accumulation of p62/SQSTM1 in RPE cells treated with bisretinoids, like the pathology observed in the Abca4 knockout mouse model for Stargardt disease [60]. This pathology parallels the elevated p62 levels observed in cultured RPE cells from AMD patients [74].

Interestingly, treatments with liver X receptor (LXR) agonists, which reduce cholesterol in RPE cells, and functional acid sphingomyelinase (ASM) inhibitors, such as the antidepressant desipramine, which lowers ceramide levels, have been shown to restore microtubule function and reinstate autophagy in RPE cells affected by bisretinoids [28, 60]. These findings suggest that controlling cholesterol and ceramide levels is essential for modulating autophagy efficiency and facilitating the elimination of cellular waste in the RPE.

The complement system, integral to immune response and inflammation, plays a significant role in the pathogenesis of AMD. Genetic mutations in complement pathway genes are linked to an increased risk of AMD, and complement proteins have been identified within drusen, the hallmark deposits in AMD-affected eyes [44]. Activation of the complement system triggers a protein cascade that forms membrane attack complexes (MAC), which create pores in cell membranes. High concentrations of MAC can destroy cells, while lower levels can induce sustained harm by elevating intracellular calcium and disrupting mitochondrial function [28].

RPE serves as a barrier against unchecked complement activation. It employs several strategies to regulate complement activity, including soluble inhibitors like complement factor H (CFH) and membrane-bound regulators such as CD46, CD55, and CD59, which work together to inhibit MAC formation [44]. CD59, which depends on cholesterol for its activity, must be efficiently recycled to maintain proper activity [75]. In Abca4 knockout mice, characterized by excessive cholesterol accumulation, CD59 is misdirected from its recycling route to lysosomal degradation, leading to increased MAC accumulation on the RPE surface [28].

Live-cell imaging has shown that RPE cells can rapidly direct lysosomes to the plasma membrane in response to complement attacks. These lysosomes merge with the membrane, sealing off forming MAC pores and blocking calcium entry [28]. Alternatively, RPE cells can engulf

MAC into endosomes and transfer them to lysosomes for degradation [76]. However, in RPE cells affected by bisretinoids, acetylated microtubules cause lysosomes to cluster near the nucleus, preventing their release and leaving MAC pores open.

The combined effect of reduced CD59 on the cell surface and impaired lysosome release results in increased intracellular calcium in the RPE, leading to mitochondrial fragmentation and the production of reactive oxygen species (ROS). These ROS further damage mitochondria, perpetuating a cycle of cellular injury and oxidative stress. Notably, reducing cholesterol with LXR agonists or ceramide with desipramine can reverse these effects by restoring CD59 on the RPE surface, facilitating lysosome release, and protecting mitochondria from complement-induced damage [55].

Further research involving AMD risk factors and human donor tissues strengthens the connection between complement activation and mitochondrial dysfunction in AMD. Exposure to AMD-related factors, such as cigarette smoke or hydrogen peroxide, reduces cell-surface complement regulatory proteins and exacerbates mitochondrial stress in RPE cells, as observed in both laboratory models and mice [77–79]. Studies on human donor eyes also reveal reduced expression of CD59 and CD46 in the RPE of AMD patients compared to healthy individuals [80, 81]. Furthermore, individuals carrying the Y402H variant of complement factor H, which is associated with AMD, show increased mitochondrial DNA damage [82]. Recent advanced imaging techniques have detected more severe mitochondrial fragmentation in the RPE of AMD donor eyes compared to healthy controls [83]. These findings collectively suggest that various AMD-related stressors, including bisretinoids, cigarette smoke, and oxidative stress, compromise the RPE's defenses against complement attacks, perpetuating a cycle of worsening mitochondrial dysfunction (Table 1).

Table 1 Classification of major genes/pathways and their role in AMD pathogenesis

Gene/pathway	Role in AMD	Reference
Cholesterol Transport (APOE, ABCA1, LIPC, and CETP)	Increased risk of AMD with variations in these genes involved in cholesterol transport within the RPE	[53, 54]
Bisretinoid Metabolism	Accumulation of bisretinoids disrupts lysosomal function and displaces cholesterol, leading to ceramide buildup	[58]
Ceramide Metabolism	Increased ceramide levels in RPE linked to AMD; BMP acts as a cofactor for ASM, the enzyme converting sphingomyelin to ceramide	[63–68]
Acid Sphingomyelinase (ASM)	Functional ASM inhibitors (desipramine) lower ceramide levels and improve RPE function	[60]
Autophagy (RB1CC1, ATG5, and ATG7)	Essential for RPE health; mutations in RB1CC1, ATG5, ATG7 impair RPE function	[69–73]
Liver X Receptor (LXR) Signaling	LXR agonists reduce cholesterol and restore autophagy in RPE cells	[60]
Complement System	Mutations in complement pathway genes (CFH) increase AMD risk; complement proteins found in drusen deposits	[28]
Complement Regulatory Proteins	RPE utilizes CFH and membrane-bound regulators (CD46, CD55, CD59) to control complement activity	[28]

Therapeutic targets in AMD: modulating gene expression and protein function for disease modification

The pathogenic pathways leading to RPE dysfunction in AMD are highly interconnected, with each exacerbating the other and perpetuating cellular damage. Understanding how various AMD risk factors drive these destructive cycles remains a significant challenge in AMD research. While AMD-associated genes impact multiple pathways, current insights into RPE injury allow for these factors to be incorporated into a unified model. This model sheds light on the different aspects of AMD onset and helps identify potential therapeutic targets within the cascade of harmful events [84]. This suggests that genetic and environmental factors act as catalysts, accelerating the mechanisms of RPE damage and pushing the natural aging process toward pathological changes.

Bisretinoids, metabolic byproducts of retinal chromophore processing, can compromise RPE health through both direct and indirect mechanisms. As a result, a range of preclinical and clinical studies are investigating strategies to inhibit their synthesis [84]. One approach involves reducing vitamin A absorption by antagonizing serum retinol binding protein 4 (RBP4) with a non-retinoid inhibitor called A1102 [85]. Another strategy uses deuterated vitamin A (ALK-001), a variant that lowers bisretinoid production while preserving the visual cycle [86]. A comprehensive clinical trial (NCT03845582) is currently evaluating the safety, pharmacokinetics, and efficacy of ALK-001 in treating GA. Other promising treatments in clinical trials include elamipretide (NCT02848313), which may protect mitochondria from molecular damage, and brimonidine tartrate (NCT02087085), which is expected to offer neuroprotective benefits [87].

Research utilizing animal models have shown potential for treatments aimed at reducing bisretinoids and improving RPE function. Antioxidants like vitamin E and iron-binding agents such as deferiprone have proven effective in reducing bisretinoid degradation, thereby protecting photoreceptors in albino Abca4 knockout mice [88]. Additionally, oral administration of ticagrelor, a P2Y12 receptor antagonist, appears to safeguard

photoreceptors in these mice by improving lysosomal function in the RPE and reducing lipofuscin buildup [89]. Additionally, RPE cells cultured from AMD patients show elevated levels of complement proteins, inflammatory indicators, and oxidative stress markers. Notably, administering nicotinamide, a form of vitamin B3, mitigates these effects, suggesting its possible therapeutic advantage [90, 91].

Genetic, epidemiological, and histological evidence strongly suggests a link between irregular cholesterol regulation and the development of AMD. Genes involved in cholesterol transport (APOE, ABCA1) and the restructuring of HDL (CETP, LIPC) are significantly correlated with AMD risk [53, 92]. Epidemiological research has also verified that elevated levels of HDL cholesterol and reduced triglyceride levels correlate with a decreased risk of AMD [54]. Interestingly, another study identified a direct relationship between serum levels of activated complement (C3d/C3), HDL cholesterol, and the incidence of AMD, suggesting potential interactions among these factors [93].

Although the exact relationship between serum HDL cholesterol and AMD is not fully understood, it is known that genes linked to both AMD and cholesterol regulation are active within the RPE [92]. This suggests that genetic variations associated with AMD may influence cholesterol transport and lipoprotein metabolism specifically in the retina. Supporting this notion, experimental evidence from mice with an RPE-specific knockout of the cholesterol transporter ABCA1 shows that these mice experience cholesterol accumulation in the RPE, progressive tissue degeneration, photoreceptor cell death, and vision impairment [57].

Thus, elevated cholesterol levels in the RPE, whether due to transport disruption or as a secondary effect of lipofuscin accumulation, are implicated in RPE damage and malfunction [60–62]. Furthermore, higher concentrations of cholesterol and cholesterol transport proteins, such as ApoB and ApoE, have been identified in drusen deposits from the eyes of AMD patients [94–96]. While the link between the size and number of drusen and AMD progression has been well-documented, the underlying processes leading to drusen formation have only recently begun to be understood.

Cutting-edge live-cell imaging techniques have shed light on the role of ApoE isoforms, protein variants with minor structural differences, in regulating RPE cholesterol levels and possibly in the formation of drusen [83]. In humans, there are three ApoE isoforms (ApoE2, ApoE3, and ApoE4), each differing by just one amino acid, which significantly influences their ability to bind lipoproteins and manage cholesterol [97–99]. These isoforms are distributed within the population, with ApoE2 at approximately 2%, ApoE3 at 78%, and ApoE4 at 14% [100]. Interestingly, these isoforms have contrasting associations with the risks of Alzheimer's disease and AMD. While ApoE4, which increases Alzheimer's risk, appears to be protective against AMD, ApoE2, protective in Alzheimer's, is linked to a higher risk of AMD [101, 102]. Live-cell imaging has demonstrated that vesicles containing ApoE2 exhibit restricted, short-range movement, in contrast to those with ApoE3 or ApoE4, which display more directed, extensive movement. This suggests that ApoE2 is less effective at transporting cholesterol compared to the other isoforms. As a result, RPE cells with the protective ApoE4 isoform maintain lower cholesterol levels, promoting proper autophagy and protecting against complement-induced mitochondrial damage. In contrast, RPE cells with the ApoE2 isoform, associated with increased AMD risk, tend to accumulate cholesterol, leading to autophagy dysfunction and heightened vulnerability to mitochondrial damage [55].

Each ApoE isoform is characterized by a distinct structural attribute: intrinsically disordered regions. These regions facilitate multiple, simultaneous, and weak interactions among the isoforms, leading to a process known as liquid-liquid phase separation. This phenomenon involves the formation of dense biomolecular clusters within the cell cytoplasm, lacking any membrane enclosure [103]. Studies in Alzheimer's disease have shown that protein mutations can alter the structure of these clusters, resulting in insoluble aggregates [104]. Such aberrant phase transitions may arise from mutations in protein, post-synthetic modifications, or environmental changes within the cell, all of which can influence protein configuration and function. Biomolecular condensates have become a significant area of interest in understanding cellular pathologies due to their role in influencing protein behavior and disease progression.

In RPE cells expressing the ApoE2 isoform, which is associated with increased AMD risk, mitochondrial damage leads to the oxidation of two cysteine residues in the protein. This oxidation enhances ApoE2's intrinsic disorder, resulting in protein aggregation. Consequently, substantial biomolecular condensates form, which may serve as precursors to drusen deposits in ApoE2-positive RPE. In contrast, ApoE4, which contains arginine residues instead of cysteines, resists oxidation and does not form large condensates. These findings reveal how a key AMD risk factor disrupts critical metabolic functions in the RPE, introducing a novel mechanism: oxidative stress-induced abnormal phase transitions, which may contribute to drusen formation [83].

Current research underscores the critical importance of regulating cholesterol balance in the retina, given cholesterol's influence on both metabolism and inflammation pathways closely linked to AMD [105]. The therapeutic potential of cholesterol-lowering medications,

such as statins (HMG-CoA reductase inhibitors), for AMD remains uncertain, as suggested by retrospective analyses [106, 107]. A preliminary study using high-dose atorvastatin over a one-year period reported a reduction in drusen in some participants [108], but further validation through larger, randomized, placebo-controlled trials is needed. Alternative strategies to reduce cholesterol levels include the use of LXR agonists, which activate LXR nuclear receptors, leading to the synthesis of cholesterol transporters like ApoE and ABCA1 [109]. While no synthetic LXR agonists have yet been approved for clinical use due to adverse effects, ongoing research may yield safer and more effective treatment options [110].

Ceramide, an intracellular signaling lipid, plays a crucial role in a wide range of cellular functions [111]. Studies using animal models of retinal diseases, such as retinitis pigmentosa, glaucoma, and AMD, have identified ceramide as a key factor in the pathogenesis of these conditions [55, 112, 113]. These investigations show that elevated ceramide levels can cause damage, inflammation, and apoptosis in photoreceptors, RPE cells, and the retinal vasculature. Notably, interventions aimed at reducing ceramide levels—whether through pharmacological agents or genetic engineering—have successfully prevented retinal degeneration in various models [112, 114]. These findings highlight the importance of tightly regulating ceramide within the retina and the potential consequences of imbalances in this system, often referred to as the "sphingolipid rheostat," on retinal health and function [113].

In RPE cells, an overabundance of ceramide, driven by increased activity of the enzyme acid sphingomyelinase (ASM), can disrupt membrane dynamics. This disruption leads to the formation of abnormal endosomes, the clustering of lysosomes around the nucleus, impaired autophagosome trafficking, and mitochondrial fragmentation. Interestingly, certain medications originally developed for other conditions have been found to inadvertently suppress ASM activity [115]. These include tricyclic antidepressants, such as desipramine and amitriptyline, as well as selective serotonin reuptake inhibitors (SSRIs) like sertraline and fluoxetine. These drugs act as mild ASM inhibitors by accumulating in lysosomes, where they block the interaction with a molecule critical for ASM activity, ultimately causing ASM to detach and degrade within the lysosome [116].

In Abca4 knockout mice, a model for a specific form of AMD, desipramine administration—either via injection or orally—effectively normalized ceramide levels in RPE cells to those observed in healthy controls. This treatment halted the formation of oversized early endosomes, prevented the breakdown of complement proteins, and suppressed the activation of the mTOR pathway, all of which are factors implicated in AMD progression [61]. Additionally, desipramine therapy restored proper autophagosome movement and autophagic flux in RPE cells challenged by bisretinoids, toxic byproducts of the visual cycle [60]. Furthermore, desipramine reinstated CD59, a crucial protein for RPE defense, on the cell surface, preventing the formation of membrane attack complexes (MAC) by the complement system and protecting RPE mitochondria from complement-induced damage [62].

Clinical data from AMD patients strongly support the targeting of ceramide as a viable therapeutic approach. First, analyses of sphingolipid levels in the blood of AMD patients have shown elevated ceramide concentrations, particularly in individuals with GA, and especially in those carrying the Y402H risk allele [68]. Second, higher ceramide levels have been detected in the RPE of eyes from AMD donors compared to healthy individuals [66, 67]. Third, a synthesis of findings from multiple studies indicates that the use of ASM inhibitors, such as desipramine, is associated with a significantly reduced risk of AMD onset [117, 118]. Finally, ceramide levels naturally increase with age in humans and are also elevated in the brains of patients with Alzheimer's and Parkinson's diseases [119, 120]. These findings suggest that disrupted ceramide regulation in non-dividing cells, such as neurons and RPE cells, may be a common factor underlying age-related neurodegenerative disorders, including AMD, and present a promising target for therapeutic intervention. Figure 3 illustrates AMD as a disease of converging cellular stress pathways, where ceramide-induced endolysosomal dysfunction, cholesterol mismanagement, and bisretinoid accumulation synergistically contribute to RPE damage and photoreceptor loss. Importantly, ceramide modulation not only stabilizes RPE cell health but also indirectly attenuates complement activation, a shared downstream effector in these pathways. Thus, targeting ceramide synthesis and lysosomal integrity presents a promising avenue to modulate multiple AMD mechanisms simultaneously, particularly in tandem with complement inhibitors or gene therapies aimed at restoring homeostatic regulators like CD59 or CFH.

Gene delivery systems and cell-based approaches in AMD therapy

Viral vector platforms: Adeno-associated virus (AAV) and lentiviral vectors for retinal gene delivery

The advancement of gene therapy for AMD hinges on two critical factors: identifying the most effective therapeutic protein and ensuring its sustained expression over an extended period. Future research will focus on refining vector design, selecting appropriate promoters, and optimizing delivery techniques while carefully managing the risks associated with the prolonged expression of proteins that inhibit angiogenesis or the complement system.

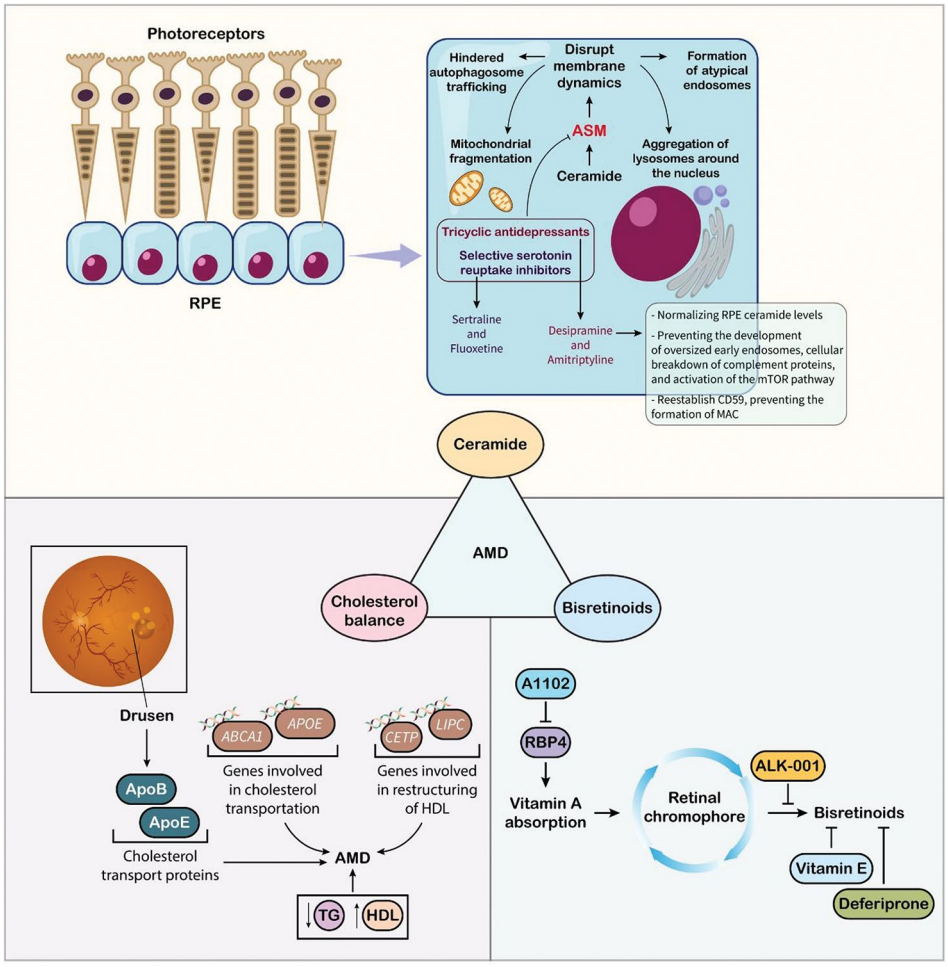


Fig. 3 Major therapeutic targets for AMD. The multifaceted diagram provided here, integrates the key molecular and cellular mechanisms underlying AMD, centering on the interplay between ceramide accumulation, cholesterol imbalance, and bisretinoid toxicity. It emphasizes how these pathways converge in the RPE and photoreceptor interface, ultimately driving retinal degeneration. Notably, it highlights therapeutic targets where ceramide modulation and complement pathway inhibition intersect to modify disease progression. At the top left, the anatomical relationship between photoreceptors and RPE cells is depicted, underscoring the functional importance of RPE in supporting photoreceptor health. Disruptions in this support system are central to AMD pathology. The upper right inset outlines the pathogenic cascade triggered by ceramide accumulation in RPE cells. Ceramide, a bioactive sphingolipid, is shown to disrupt endolysosomal and mitochondrial function via hindered autophagosome trafficking, mitochondrial fragmentation, disrupted membrane dynamics, formation of oversized, atypical endosomes, and aggregation of lysosomes around the nucleus. This cascade promotes chronic cellular stress, inflammation, and complement activation, creating an environment conducive to AMD progression. Acid sphingomyelinase (ASM) is a key enzyme that catalyzes ceramide production from sphingomyelin, serving as a central regulatory node. Therapeutically, tricyclic antidepressants (e.g., desipramine, amitriptyline) and SSRIs (e.g., fluoxetine, sertraline) inhibit ASM activity, thereby reducing ceramide synthesis. Therapeutic outcomes of ASM inhibition include normalization of ceramide levels, prevention of oversized endosome formation and lysosomal dysfunction, stabilization of the CD59 complement inhibitor, reduction in MAC (membrane attack complex) formation, and inhibition of aberrant mTOR signaling. This suggests a critical therapeutic intersection between ceramide modulation and complement inhibition, as ceramide-induced lysosomal stress may initiate or amplify complement activation, a known pathogenic feature in AMD. The lower left panel segment links cholesterol dysregulation with drusen, a hallmark of early AMD. Drusen are extracellular lipid-protein aggregates that accumulate between the RPE and Bruch's membrane. Cholesterol transport proteins (ApoB, ApoE) and related genes (ABCA1, APOE, CETP, LIPC) regulate lipoprotein metabolism and high-density lipoprotein (HDL) remodeling. Dysregulation of these pathways leads to altered triglyceride (TG) and HDL levels, accumulation of lipids in the sub-RPE space, and recruitment and activation of immune components, including complement factors. Although not directly shown in this panel, complement activation is facilitated by lipid and debris accumulation, again linking to ceramide-induced endolysosomal stress and MAC formation. The final panel (lower right panel) focuses on bisretinoids, toxic byproducts of vitamin A metabolism that accumulate within the RPE as lipofuscin. These compounds contribute to oxidative damage and cell death. RBP4 (Retinol Binding Protein 4) facilitates vitamin A transport, which can be targeted by A1102, reducing substrate availability for bisretinoid formation. Therapeutic agents such as ALK-001 (a deuterated form of vitamin A) slows bisretinoid accumulation. In addition, Vitamin E and deferiprone act as antioxidants or iron chelators, mitigating bisretinoid-induced oxidative stress. Though bisretinoid toxicity is mechanistically distinct, it converges with ceramide and cholesterol pathways at the level of RPE degeneration and complement activation, particularly as dying RPE cells can release DAMPs (damage-associated molecular patterns) that further trigger inflammation and complement cascades

Table 2 Characteristics of major viral gene delivery systems for AMD therapy

Feature	Adenoviral Vectors	AAV Vectors	Lentiviral Vectors
Payload Size	Large (up to 35 kb)	Small (4.5-5.0 kb)	Medium (up to 10 kb)
Cell Specificity	Broad	Varied by serotype (e.g., AAV2 targets retina)	Broad
Episomal or Integration	Primarily Episomal	Primarily Episomal	Can Integrate
Infects Dividing/Non-Dividing Cells	Both	Both	Both
Immune Response	High (early vectors), Lower (later generations)	Low	Moderate
Genotoxicity Risk	Low	Low	Potentially Higher (compared to AAVs)
Advantages	Large payload, efficient infection	Low immune response, tissue specific options	Integration possible, infects both cell types
Disadvantages	High initial inflammatory response, limited tissue specificity	Limited payload size, pre-existing immunity possible	Moderate genotoxicity risk
Suitability for AMD Gene Therapy	Limited due to potential inflammation	Currently the leading candidate	Potential, but safety concerns require further research
References	[122–125]	[126, 132–134]	[127, 128, 130, 131]

Over the past two decades, a wide variety of viral and non-viral methods for delivering genetic material into cells have been developed. The most extensively studied viral vectors include adenovirus, adeno-associated virus (AAV), and lentivirus (Table 2). The choice of vector is crucial and depends on the specific application, considering factors such as tissue specificity (tropism), payload capacity (cloning size), and safety concerns, including the risks of inflammation and oncogenesis (genotoxicity/insertional oncogenesis) [22, 121].

The most advanced adenoviral vectors, known as helper-dependent vectors, can transport a substantial genetic payload (around 35 kb) and infect a broad range of cell types, including non-dividing cells. Typically, the viral DNA remains episomal, meaning it does not integrate into the host genome, which minimizes the risk of insertion. However, earlier versions of adenoviral vectors were associated with inflammatory reactions [122, 123]. More recent iterations, including helper-dependent adenoviral vectors, are less likely to provoke immune responses and are considered the safest among adenoviral platforms [124, 125].

AAV vectors are particularly attractive for gene therapy due to their minimal immune response, making them suitable for a wide range of human diseases. Like adenoviral vectors, AAV vectors can infect non-dividing cells, and its genetic material predominantly remains episomal, avoiding integration into the host DNA (episomal). A major advantage of AAV vectors is the availability of various subtypes, each with a predilection for infecting specific tissues. For example, AAV2 is known to target muscle, liver, CNS, and retina, whereas AAV8 is more specific to liver, retina, CNS, pancreas, and heart [126].

Lentiviral vectors, derived from viruses such as HIV-1 or EIAV, can carry up to 10 kb of genetic information [127, 128]. These vectors are particularly advantageous when the integration of the delivered genetic material into the host genome is required. Lentiviral vectors have been widely used to modify hematopoietic stem cells [129]. Recently, they have gained popularity due to their ability to infect both dividing and non-dividing cells, and they present a lower risk of insertional oncogenesis compared to gamma-retroviruses [130]. Additionally, non-integrating lentiviral vectors have been engineered to further reduce the risk of genotoxicity [131].

Currently, AAV vectors are the leading viral vectors used in gene therapy for retinal diseases. Their suitability for retinal applications stems from several key characteristics, including their lack of integration into the host genome, minimal potential to trigger inflammation, low retinal toxicity at appropriate doses, non-pathogenic nature, ability to target non-dividing cells, and strong safety profile in human clinical trials [132]. However, AAV vectors do have limitations. Their cloning capacity is relatively small (4.5-5.0 kb), and there is a risk of immune system clearance in patients who have been previously exposed to the AAV virus [133]. Fortunately, this risk is relatively low when targeting the retina, which is considered an immune-privileged site [134].

Once talking about therapeutic strategies against AMD, it is worth considering that AAV-based gene therapy approaches for AMD and inherited retinal diseases (IRDs) require fundamentally different strategies due to distinct disease mechanisms and patient populations. First, AMD involves complex interactions between genetic susceptibility such as complement factor H variants, environmental factors, and age-related oxidative stress [135, 136]. Thus, therapeutics must focuse on pathway modulation (VEGF suppression, complement inhibition) rather than single-gene correction. Requires sustained expression due to chronic disease progression. On the other front, IRDs are principally caused by >280 identified mutations in genes like RPE65, ABCA4, and USH2A. Examples include LCA and retinitis pigmentosa

[135, 136], and thus gene replacement strategy aims to restore functional protein expression through retinal delivery of wild-type transgenes, as demonstrated by Luxturna® (voretigene neparvovec) [135].

With specific modifications for AMD and IRDs, vector design specifications for retinal gene therapies must be adapted to the disease context. To minimize off-target effects during prolonged anti-VEGF treatment, cell-specific promoters are necessary for AMD in order to ensure targeted expression in Müller glia or RPE cells. Furthermore, dual transgene systems, which combine VEGF suppression with complement pathway modulation, for example, through CR2-fH hybrid constructs, are showing promise. On the other hand, IRD approaches frequently call for more extensive expression profiles, which makes ubiquitous synthetic promoters like CAG perfect for attaining pan-retinal gene expression in conditions that impact various retinal cell types. Further optimization is achieved through capsid engineering; novel AAV variants like AAV2-7m8 and AAV44.9 have demonstrated enhanced retinal penetration, facilitating efficient subretinal delivery to photoreceptors [135, 136].

The development of retinal gene therapies is also hampered by immunological issues, as there are notable distinctions between cohorts with IRD and those with AMD. Neutralizing antibodies against widely used AAV serotypes, including AAV2 and AAV8, are highly prevalent in older adults with AMD, with seroprevalences ranging from 40 to 60%. To avoid humoral immune responses, this calls for either pretreatment immunosuppression or the use of modified capsids such as AAV8BP2. Children with IRDs usually have baseline immunity levels below 20%, which makes them more responsive to direct subretinal gene delivery without requiring significant immunomodulation. The localized nature of ocular administration and the relatively immature immune systems in younger individuals also contribute to reduced systemic immune risks in this population [135, 136].

Innovative approaches that specifically address the difficulties of AMD have been made possible by pathophysiology-driven engineering. One such development is the creation of protease-activated vectors, which allow targeted transgene activation only in diseased tissues by taking advantage of the elevated levels of matrix metalloproteinase-9 (MMP-9) in neovascular AMD. Simultaneously, dual-function transgenes are being developed to provide combined therapeutic effects. For instance, combining anti-inflammatory cytokines like IL-10 with anti-angiogenic agents like sFLT-01 can reduce inflammation and inhibit the growth of pathological vessels. Methylation-resistant promoters have also been added to guarantee long-term therapeutic benefit. This is important for the chronic treatment of AMD because it permits long-term gene expression in the metabolically stressed RPE [135, 136].

Overcoming the cargo limitations of AAV vectors is a primary focus of gene therapy development for IRDs. By dividing the genetic payload among two AAV vectors that reconstitute the full-length transcript within the target cells, trans-splicing systems, which allow the delivery of large genes, like ABCA4, implicated in Stargardt disease, offer a promising remedy. Moreover, endogenous microRNA-responsive elements are used to improve transgene expression precision. These elements use retinal-specific miRNA profiles to suppress off-target expression in non-relevant cell types, which improves safety and therapeutic specificity. These distinctions underscore the need for disease-specific vector optimization, as highlighted in recent reviews of ocular gene therapy [136]. While IRD therapies benefit from well-defined genetic targets, AMD requires multilayer engineering to address complex pathophysiology and age-related biological barriers. Ongoing clinical trials continue to refine these approaches through capsid evolution and immune evasion strategies [135].

Table 3 Non-viral gene delivery systems for AMD therapy

Feature	Lipid Nanoparticles	Exosomes	Membrane Hybrid Exosomes (HEs)
Composition	Synthetic lipids	Natural lipid bilayers derived from cells	Fusion of liposomes and exosomes
Cargo Capacity	Limited (small RNA, CRISPR/Cas9)	Limited (proteins, microRNAs)	Larger than exosomes (CRISPR/Cas9)
Delivery Mechanism	Endosomal escape and nuclear entry required	Utilize natural cellular pathways	Utilize natural cellular pathways
Advantages	Low cytotoxicity, low immunogenicity	Biocompatible, targeted delivery potential	Larger cargo capacity than exosomes
Disadvantages	Endosomal escape and nuclear entry limitations	Limited cargo capacity	Lower efficiency for CRISPR/Cas9 delivery
Suitability for AMD Gene Therapy	Promising for delivering small RNA	Promising for delivering proteins and microRNAs	Potential for in vivo gene editing, requires further research
References	[137–141]	[142–145]	[146, 147]

Non-viral delivery systems: lipid nanoparticles and exosomes for ocular gene transfer

Lipid nanoparticles (LNPs), tiny spheres that mimic cell membranes, are emerging as a leading non-viral vector for gene therapy in clinical applications (Table 3) [137]. They are favored over other non-viral vectors due to their reduced cytotoxicity and immunogenicity. Research by

Finn et al. and Sung et al. has demonstrated the efficacy of LNPs in delivering CRISPR/Cas9 elements for gene modification, achieving high expression in target cells with minimal cellular toxicity [138, 139]. These findings highlight the potential of LNPs to address retinal disorders and other posterior ocular conditions [140, 141]. Their exceptional ability to encapsulate genetic material and penetrate target cells makes them particularly suitable for gene delivery to retinal cells.

One of the major advantages of LNPs is the absence of viral elements, which reduces safety concerns and the risk of immune reactions. However, several challenges remain. First, after entering the cell, the nanoparticles must escape from endosomes to release their contents.

Additionally, the Cas9 complex, essential for gene editing, must successfully navigate to the nucleus [142]. As a result, the overall efficiency of CRISPR/Cas9 delivery via LNPs is somewhat limited. The success of this delivery also hinges on variables such as the type of target cell and the nature of genetic cargo, requiring careful customization of the lipid formulation for each specific case.

Exosomes offer another promising avenue for gene delivery. These small vesicles, composed of lipid bilayers, play a crucial role in intercellular communication and show great promise for both disease diagnosis and therapy. Their functionality and composition are influenced by their cellular origin, which dictates their effects based on the originating cell type. Evidence indicates that exosomes from RPE cells under oxidative stress can serve as vehicles for proteins and microRNAs [143]. Investigations by Kang et al. and Biasutto et al. have revealed that exosomes can transport hydrophobic receptor proteins from retinal cells to the vitreous cavity [144]. Similarly, Hajrasouliha et al. demonstrated that exosomes derived from RPE cells associated with choroidal neovascularization can be targeted to specific cells involved in the condition, offering a promising approach for treating AMD [145]. Despite their potential as drug carriers, exosomes face limitations in housing large nucleic acids required for CRISPR/Cas9 systems.

Recent studies are exploring membrane hybrid exosomes (HEs), formed by fusing liposomes with exosomes. Unlike standard exosomes, HEs can accommodate larger genetic constructs, such as CRISPR/Cas9 vectors, enabling potential in vivo gene editing. Due to the porous nature of choroidal vessels and the size of HEs (approximately 200 nm), these hybrids may be capable of crossing vessel walls and reaching target endothelial cells. This approach could offer a safer alternative to traditional intravitreal injections, which carry infection risks. However, further research is required to optimize local delivery methods for these nanoscale exosomes, particularly beyond the ocular setting. Encapsulating the CRISPR/Cas9 system within exosomes poses significant challenges compared to other carriers, as plasmid DNA incorporation via electrofusion typically results in low efficiency [146, 147]. Developing targeted strategies for disease-specific cellular therapies is essential for advancing exosome-based therapeutics.

Stem cell-based approaches for AMD treatment

Stem cells are defined by two key characteristics: [1] the ability to proliferate and produce identical multipotent stem cells indefinitely while maintaining their undifferentiated state, and [2] the potential to differentiate into various cell types [148]. Human embryonic stem cells (hESCs), which are pluripotent, were first successfully isolated and cultured in 1998 by Thomson et al. (1998). Following this breakthrough, Klimanskaya et al. (2004) developed the foundational protocol for deriving RPE-like cells from hESCs. This innovation sparked considerable optimism for the potential of an unlimited supply of RPE cells for treating AMD [148]. Over the past decade, various research groups have employed different methodologies to generate RPE cells from stem cells, with varying degrees of success. Nevertheless, recent technical and regulatory advancements have made stem cell-based therapies for AMD increasingly feasible. These advancements encompass [1] the derivation of RPE cells from hESCs [2], progress in utilizing induced pluripotent stem cells (iPSCs) [3], the employment of stem-cell-derived retinal progenitor cells to compensate for photoreceptor loss, and [4] the initiation of clinical trials employing stem-cell-derived RPE for retinal degenerative conditions. Mounting evidence suggests that the short-term safety of stem-cell-derived RPE implantation poses no significant concern [149]. However, several key challenges remain for the clinical application of stem cell-based treatments for AMD, including: [1] the development of commercially viable, cost-effective, reliable, and robust sources of RPE and retinal progenitors; [2] the refinement of techniques for delivering stem cell-derived RPE into the subretinal space; [3] ensuring the long-term viability and functionality of the transplanted cells; [4] confirming the long-term safety of the procedure, particularly the absence of tumor formation years post-implantation; and [5] devising methods to harness the immunoregulatory properties of stem cell-derived RPE to reduce the risk of immune rejection.

It remains to be determined whether therapies involving stem cell-derived RPE or retinal progenitor cell (RPC) replacement merely decelerate the progression of retinal degeneration or actively enhance vision by integrating into the retina and restoring its function. The transplantation of hESC-RPE into the subretinal space in animal models of retinal degeneration has demonstrated the ability to preserve deteriorating photoreceptors and improve vision [150–152], providing proof of concept that

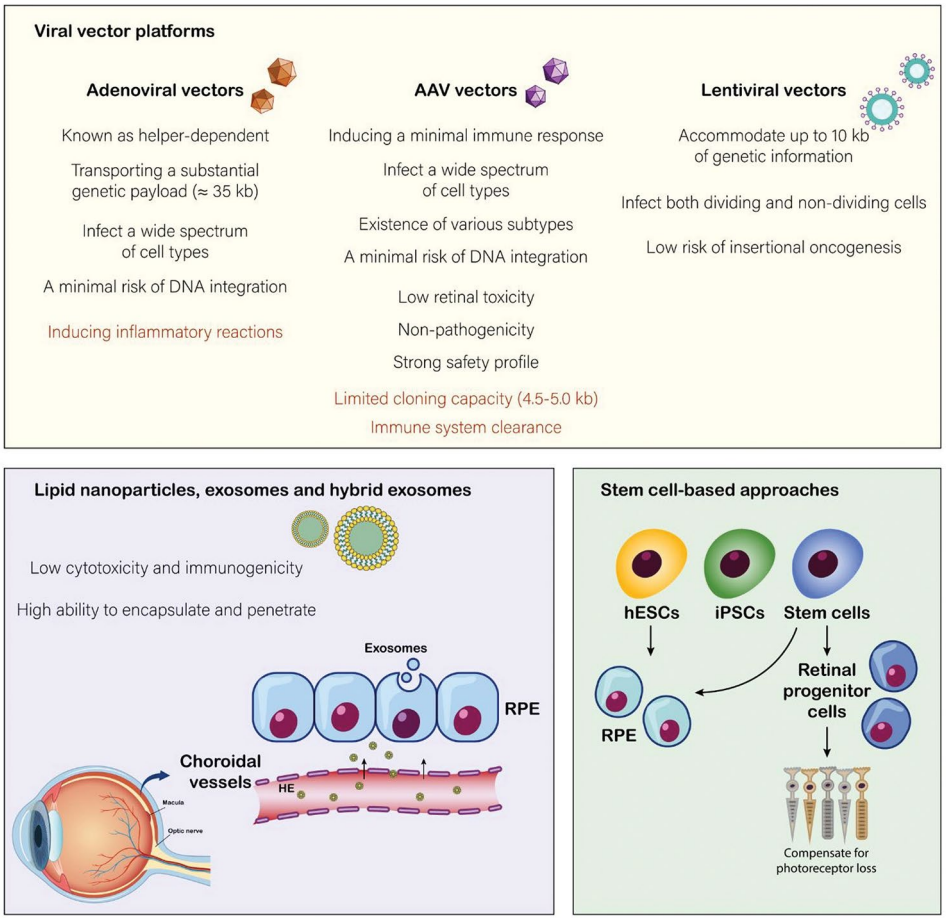


Fig. 4 Gene Delivery Systems and Advanced Therapeutic Strategies for AMD therapy. A range of contemporary approaches under investigation for the treatment of retinal degenerative diseases, particularly AMD, is seen in this figure. The top panel details viral vector platforms, including Adenoviral, Adeno-Associated Viral (AAV), and Lentiviral vectors, highlighting their respective advantages and limitations regarding genetic payload capacity, immunogenicity, cell tropism, and safety profiles. At the bottom left panel, non-viral delivery methods utilizing lipid nanoparticles, exosomes, and hybrid exosomes are illustrated, emphasizing their low cytotoxicity and high encapsulation and penetration capabilities across the retinal pigment epithelium (RPE) and into the choroidal vessels. The bottom right panel outlines stem cell-based therapies, showcasing the potential of human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), and other stem cell sources to differentiate into retinal progenitor cells for transplantation and compensation of photoreceptor loss

hESC-RPE transplantation could be a viable therapeutic approach. The optimization of RPE transplantation procedures has led to the emergence of two distinct therapeutic strategies: [1] the introduction of a cell suspension comprising non-polarized hESC-RPE cells into the subretinal space, facilitating integration with the host retina, and [2] the transplantation of polarized sheets of hESC-RPE, which improves safety and clinical outcomes due to the reliance of normal RPE function on the specialized characteristics of its apical and basal domains. iPSC-RPE cells, derived from a patient's own cells, exhibit authentic RPE characteristics, including the ability to interdigitate with photoreceptor outer segments and provide support [153]. Stem cell-derived RPCs have also been proposed to replace lost photoreceptors, either independently or in conjunction with hESC-RPE cell transplantation [154]. While fetal umbilical cord blood cells and hematopoietic stem cells are multipotent and are being explored for the treatment of retinal degenerative diseases [155, 156], they have not shown as much promise as the use of hESC-RPE, iPSC-RPE, and RPC therapies to date (Fig. 4).

Gene therapy for AMD is rapidly progressing with a wave of technological advancements with the objective of addressing the disease's chronic and multifaceted nature. Recent preclinical and clinical advances are focused on overcoming underlying challenges such as sustained pathway modulation, targeted cellular delivery, and combinatorial therapeutic strategy integration.

One of the growth opportunities is combinatorial therapy dual-vector systems. For example, anti-C3 and anti-VEGF fusion proteins delivered by AAV.CB. anti-C3-anti-VEGF in murine models was demonstrated to

reduce serum levels of C3 by 27% and efficiently inhibit VEGF. Two-way-path therapy in this manner provides for concurrent antagonism of both complement dysregulation and neovascularization, with a resultant slowing of geographic atrophy progression in preclinical studies following subretinal administration. The second is the application of an R100 capsid to co-deliver aflibercept with a VEGF-C-targeting miRNA in the form of the 4D-150 therapy. This system could deliver a 99% reduction in grade IV choroidal neovascularization (CNV) lesions in primate models, showing the potential for combining anti-angiogenic drugs with RNA-based modulators. AAV7m8-based vectors, such as those used in the ADVM-022 program, have also demonstrated the feasibility of intravitreal delivery, with 67% of patients remaining injection-free at 2.5 years for phase 1 trials. Notably, the R100 capsid supported a 98% reduction in CNV area through dual transgene expression, and this is a significant step towards long-term, non-invasive treatment [157–159].

Precision targeting has also been enhanced through cell-specific promoters. Synthetic RPE-specific promoters have outperformed the endogenous BEST1 promoter eightfold with cell specificity maintained and enhanced transgene expression. Hybrid promoter constructs that fuse viral enhancer elements, e.g., CMV immediate early fragments, onto RPE transcription factor-responsive elements (TFREs) have also emerged with similar efficacy but 15% smaller size compared to classic CMV promoters. To ensure long-term stable transgene expression, especially in metabolically stressed RPE cells, methylation-resistant promoter constructs have been engineered to be resistant to epigenetic silencing, a necessary feature to address the chronic nature of AMD [160, 161].

These gene therapy vectors also incorporate immune management strategies to address age-dependent immune responses. Topical steroids, for instance, are being used to minimize anterior chamber inflammation associated with AAV7m8 vectors, and codon optimization strategies are being employed to minimize HLA class I presentation and the risk of cytotoxic immune activation.

Together, these advances comprise a strategic shift toward multifactorial treatment in line with AMD's complex pathophysiology. By combining dual pathway targeting, capsid engineering, and promoter optimization, researchers aim to overcome both biological and immunological challenges inherent to aged retinal tissue. Ongoing clinical trials have the potential to determine whether these next-generation gene therapy platforms can successfully bridge preclinical promise and sustained clinical efficacy [121, 162–165].

Current status of gene therapy clinical trials for AMD

Gene therapy candidates and target genes for AMD: overview of completed and ongoing clinical trials

Most gene therapy clinical trials for AMD have focused on the neovascular variant (nAMD) [166]. Pigment epithelial-derived growth factor (PEDF), a protein with multiple roles, including promoting neuron survival and inhibiting angiogenesis has been identified as a potential therapeutic agent [167, 168].

In a phase 1 trial conducted in 2006, researchers evaluated the administration of PEDF via a one-time intravitreal injection of an AAV vector in 28 patients with nAMD. Patients who received a lower dosage (under 10^8 particle units) experienced a deterioration in vision and an increase in macular neovascularization (MNV) lesions. Conversely, those administered a higher dosage (at least 10^8 particle units) were more likely to experience disease stabilization, suggesting a possible dose-dependent effect [169].

Adverum Biotechnologies, previously known as Avalanche Biotechnologies, developed AVA-101, a gene therapy that employs a modified AAV2 vector to deliver the gene for soluble human vascular endothelial growth factor receptor 1 (sFlt-1). AVA-101 was assessed in a phase 1/2a clinical trial. In phase 1, eight patients received a single subretinal injection of AVA-101, along with initial standard injections of ranibizumab, followed by additional injections as required. Over 36 months of follow-up, AVA-101 was well-tolerated, with no significant safety concerns reported [166].

The phase 2a results, disclosed in 2015, met the primary safety endpoints, affirming the ocular and systemic safety of AVA-101. However, the data on visual and anatomical outcomes were inconclusive. Although the average vision improvement in the AVA-101 group was marginally better than that of the ranibizumab control group, this difference was likely attributed to the more advanced disease stage in the control group. Notably, 43% of patients in the AVA-101 group experienced either maintained or improved vision with no more than two additional ranibizumab injections, compared to only 9% in the control group. In terms of retinal structure, OCT scans revealed a minor increase in central retinal thickness in the AVA-101 group, whereas the control group exhibited a decrease. Despite these encouraging outcomes, Adverum decided not to proceed with a phase 2b trial for AVA-101 at the end of 2015 [166].

ADVM-022 is an innovative gene therapy for nAMD, designed to minimize the frequency of anti-VEGF injections. It employs an AAV7m8 capsid to transport an aflibercept expression cassette, enabling targeted cells to continuously synthesize this anti-angiogenic factor. Preclinical trials in primates showed sustained aflibercept

production and reduced choroidal neovascularization, leading to the initiation of a phase 1 trial (OPTIC trial). This open-label study includes nAMD patients who have previously undergone extensive anti-VEGF therapy. Initial findings are promising, indicating a significant reduction in the number of anti-VEGF injections required annually in both the high-dose and low-dose groups. The primary adverse effect observed was eye inflammation, particularly in the anterior segment of the eyes in the high-dose group, with some patients requiring long-term topical anti-inflammatory treatments. Inflammation was less frequent in the low-dose group, suggesting a potential dose-related response [166].

Another promising gene therapy, RGX-314, is being developed by REGENXBIO in collaboration with AbbVie for nAMD. Based on the AAV8 vector, this therapy delivers a gene encoding a monoclonal antibody fragment akin to ranibizumab, via subretinal or suprachoroidal injections. This antibody fragment binds to VEGF-A, inhibiting neovascularization and potentially reducing the need for regular intravitreal injections.

Both ADVM-022 and RGX-314 are emerging as promising gene therapy alternatives for individuals with nAMD, striving to alleviate the burden of recurrent injections by enabling the continuous production of anti-VEGF agents within the eye. Although still in the preliminary phases of clinical trials, the initial outcomes are encouraging and highlight the transformative potential of gene therapy in nAMD treatment.

RGX-314 is currently being assessed across seven active clinical trials, as listed on ClinicalTrials.gov. In 2023, REGENXBIO reported promising preliminary results from these studies, with 46 participants showing no significant adverse effects linked to the therapy. While long-term data are still awaited, early indicators of efficacy include maintained or improved visual acuity, better retinal structure, and a reduction in the frequency of anti-VEGF injections required by patients receiving higher doses. These early outcomes suggest that RGX-314 could serve as a safe and effective alternative to regular anti-VEGF injections for individuals with nAMD. The confirmation of these results depends on continued data collection from ongoing trials, but the progress of RGX-314 underscores the potential of gene therapy in managing nAMD [166].

In a recent investigation, 30 individuals with nAMD received high doses of RGX-314. At the six-month evaluation, researchers noted several promising outcomes, further highlighting the potential of this therapeutic approach. Every participant receiving the high doses exhibited consistent levels of the anti-VEGF protein fragment in their eyes, indicating stable administration and expression of the gene therapy. Moreover, these patients either maintained or improved in best-corrected visual acuity (BCVA) and central retinal thickness (CRT), suggesting the therapy helps preserve retinal integrity and function. Most importantly, the need for post-treatment anti-VEGF injections was significantly reduced, underscoring the potential of RGX-314 to reduce the treatment burden for nAMD patients [166]. While RGX-314 shows positive outcomes, it represents just one of many strategies currently being explored. According to ClinicalTrials.gov, multiple active trials are focusing on RGX-314, although only one trial has published findings thus far [166].

Another innovative gene therapy being developed for nAMD is RetinoStat, created by Oxford BioMedica. This therapy employs a lentiviral vector to deliver genes encoding endostatin and an angiotensin protein. The anti-angiogenic properties of endostatin, combined with the involvement of the renin-angiotensin-aldosterone system in AMD, form the foundation of this therapeutic approach. An ongoing phase 1 clinical trial (NCT01301443) is evaluating the safety of a one-time subretinal injection in 21 participants with advanced nAMD resistant to current treatments, using a dose-escalation approach to determine the optimal dose. The active phase 1 trial of RetinoStat highlights the dynamic and evolving field of gene therapy for nAMD. While RGX-314 shows great promise, the exploration of diverse gene therapy methodologies, such as RetinoStat, broadens the scope of potential future therapies [170].

In addition to ADVM-022 and RGX-314, drug-tunable Flt23k gene therapy presents another novel approach for treating retinal neovascularization. Flt23k is a decoy receptor that binds VEGF and is fused with the destabilizing domain (DD) of Escherichia coli dihydrofolate reductase (DHFR). This fusion protein remains degraded unless stabilized by trimethoprim (TMP). After intravitreal injection of a self-complementary AAV vector (scAAV) encoding DHFR(DD)-Flt23k, TMP administration enables tunable VEGF suppression, as demonstrated in a rat model of oxygen-induced retinopathy (OIR) [171]. This strategy presents a controlled therapeutic approach for managing ischemia-induced retinal neovascularization. (Table 4)

Gene therapy has also been shown to be a hopeful treatment option for geographic atrophy (GA) caused by AMD, particularly through targeting the dysregulation found within the complement system, which is a key player in retinal degeneration. Of the therapies being researched, GT-005 has garnered much attention through its gene augmentation strategy aimed at restoring complement regulation through the delivery of complement factor I (CFI). GT-005 utilizes an AAV2 vector for delivering human CFI to the retinal tissues, with the goal of restoring equilibrium to the hyperactive complement cascade implicated in GA progression. Preclinical

Table 4 Clinical trials on AMD gene therapy

Gene Therapy Trial	Year	Trial Phase	Details	Outcomes	References
PEDF via AAV Vector	2006	1	Explored PEDF administration in nAMD patients.	Lower doses led to vision deterioration, while higher doses suggested disease stabilization.	[169]
JNJ-81,201,887 (Janssen)	2023 (Active, Not Recruiting)	2	Intravitreal injection of AAV2 vector with sCD59 gene for nAMD and GA.	Well-received, consistent reduction in GA lesion growth rates.	NCT05811351
RGX-314 for nAMD (by REGENXBIO and AbbVie)	2023	Various (see ClinicalTrials.gov)	AAV8 vector delivering anti-VEGF monoclonal antibody fragment.	Promising initial results; reduced injection frequency; ongoing trials.	NCT05407636 NCT04704921 NCT04514653 NCT04832724 NCT03066258
ADVM-022 by Adverum Biotechnologies	2022 (Active, Not Recruiting)	2	AAV7m8 capsid delivering aflibercept expression cassette in nAMD patients.	Reduced need for anti-VEGF injections; eye inflammation as an adverse effect.	NCT05536973
FT-003	2023	1	Not specified	Results not yet published.	NCT05611424
BD311 (AAV-CFI)	2021	Early phase 1 (Recruiting)	Not specified	Results not yet published.	NCT05099094

studies in non-human primates demonstrated favorable pharmacokinetics, with sustained intraocular expression of CFI up to 1,437 ng/mL at higher dosages, and no evidence of systemic toxicity. These findings initially justified clinical development. However, Phase 2 HORIZON trials did not demonstrate substantial clinical effectiveness. Although gene delivery was achieved, GT-005 was not sufficiently capable of inhibiting complement activity or creating detectable anatomical or functional improvement in patients. The inability of therapeutic response combined with the complexity of treating long-term complement activation in late AMD led to program discontinuation in 2023 [172].

JNJ-81,201,887 is another investigational gene therapy for geographic atrophy in age-related macular degeneration, utilizing an AAV vector to deliver soluble CD59 (sCD59) to inhibit membrane attack complex (MAC) formation to restrict retinal damage. Results of Phase 1 trial (2024) showed no serious adverse effects in 17 patients for 24 months at all doses, establishing its safety. However, efficacy results on GA progression are still awaited. Major limitations include the failure to sufficiently inhibit upstream complement components, including C3 and C5, and the uncertain long-term stability of sCD59 expression in the aging retina. While early safety results are reassuring, more data are needed to determine both the clinical benefits and the extended efficacy [173, 174].

While gene therapy offers theoretical advantages for GA, clinical outcomes have yet to surpass conventional complement inhibitors. Further research is needed to optimize vector design, immune tolerance, and biomarker-guided patient selection.

Challenges in AMD gene therapy: roadblocks to clinical translation

While gene therapy holds considerable promise for treating AMD), several critical limitations and risks must be acknowledged. Although emerging safety and efficacy data for AMD gene therapy are encouraging, the lack of long-term results raises concerns about potential enduring side effects and the sustained impact of these treatments over time [175]. Additionally, the process of accurately delivering the therapeutic gene to the designated retinal cells remains complex. Despite significant progress in refining intraocular delivery techniques, a universally successful and consistent method for targeting retinal cells across all patients has yet to be established [166].

A significant challenge arises from the immune system's response to viral vectors used in gene therapy, which may be recognized as foreign, triggering an inflammatory reaction. The severity of this inflammation can vary from mild to severe, influenced by factors such as dosage, delivery method, the type of viral vector, the promoter, and the specific gene delivered. For example, adenovirus vectors tend to provoke a stronger inflammatory response compared to adeno-associated virus (AAV) vectors. Furthermore, the presence of neutralizing antibodies (NAbs) in a patient's blood may hinder the viral vector's ability to effectively deliver the therapeutic gene, with the prevalence of these antibodies varying across different AAV serotypes [176, 177].

Ensuring the safety of the introduced gene is another critical factor. Certain genes, such as green fluorescent protein (GFP), commonly used in research, have been shown to be harmful to cells. [165] Additionally, evidence suggests that subretinal injections may result in less inflammation compared to intravitreal injections. While inflammation following intravitreal gene therapy typically

responds well to treatment, the potential for these complications must still be considered. In rare cases, retinal atrophy has been observed following subretinal gene therapy [178, 179].

The intricate nature of gene therapy procedures and their high costs raise questions about the practicality of widespread application, potentially leading to ethical concerns over treatment accessibility [180]. These challenges emphasize the importance of continuing research to refine delivery methods, mitigate immune responses, and reduce costs to make these therapies more broadly available.

In summary, gene therapy offers a hopeful avenue for the management of AMD, but the associated challenges and risks underscore the necessity for ongoing research and development. Ensuring the safety, efficacy, and accessibility of these therapies will be critical to their success as a long-term solution for AMD.

Immunogenicity and safety concerns: host immune responses to viral vectors and transgene products

The immune system serves as a crucial defense against infections, comprising two primary components: innate and adaptive immunity. The innate immune system reacts rapidly and non-specifically, while the adaptive system responds more slowly, with precision and memory for subsequent exposures. Gene therapy utilizes viral vectors to transport therapeutic genes into cells. These vectors are engineered to be non-replicating and benign, yet they may still provoke unforeseen immune responses. The potential immune reactions to gene therapy vectors include pre-existing immunity, where antibodies from prior infections neutralize the vector before gene delivery; the innate immune response, where recognition of viral elements by the immune system may trigger inflammation, potentially diminishing therapeutic efficacy; and the adaptive T cell response, where dendritic cells (DCs) present viral antigens to T cells, activating cytotoxic T cells (CTLs) that eliminate vector-infected cells. Simultaneously, helper T cells may be activated, potentially leading to antibody production [181].

Historically, the immune responses associated with high-dose gene therapies, particularly for conditions like muscular dystrophy, were not thoroughly studied. However, recent research has uncovered novel immune-related toxicities associated with these high doses, which may arise from the vector, product impurities, or the effects of the introduced gene. For instance, high doses of AAV9 vectors can activate the complement system, an immune pathway responsible for neutralizing foreign entities. At elevated doses, viral particles may coat cell surfaces, making them susceptible to antibody attacks. Moreover, gene therapy trials have reported broader toxicities, such as thrombocytopenia (a reduction in platelet count), and CD8 + T cell responses that could potentially damage various organs or the central nervous system. Further research is imperative to identify and mitigate these intricate immune responses, a critical step toward ensuring the safety and efficacy of high-dose gene therapy [181].

Lentiviral (LV) vectors, derived from HIV, have a unique ability to deliver genes into both dividing and non-dividing cells. These vectors consist of an enveloped structure and a single-stranded RNA genome, which is reverse-transcribed into DNA within the host cell. This DNA is then transported to the nucleus and integrated into the host genome, ensuring the long-term expression of the transgene if the host cell survives. LV vectors are commonly used for gene transfer to hematopoietic stem cells (HSCs) to treat genetic diseases and to T cells for chimeric antigen receptor (CAR)-T cell therapy in cancer. They are also being developed for direct in vivo gene transfer, such as to the liver, and integration-deficient versions have been engineered. A novel application of LV vectors involves in vivo gene transfer to DCs, a technique gaining traction in vaccine development. LV vectors are typically pseudotyped with the vesicular stomatitis virus (VSV)-G protein, which allows efficient infection across a broad range of cells. However, VSV-G pseudotyped LV vectors do not target B cells, prompting the development of CD20-specific envelopes, which combine an anti-CD20 single variable fragment with a measles virus envelope protein for targeted infection. Specific B and T cell-targeting envelopes have also been engineered [182–185]].

Despite the relatively low pre-existing immunity to LVs in humans, the effectiveness of in vivo hepatic gene transfer using LV vectors is challenged by obstacles such as phagocytosis. Integrating the human phagocytosis inhibitor CD47 into the LV membrane reduces uptake by phagocytic cells and enhances distribution to hepatocytes [186]. Another challenge involves the production of type I interferon (T1 IFN), as mice lacking T1 IFN signaling demonstrate a marked increase in transduced hepatocytes. Inhibiting IFN production with pharmacological agents, such as dexamethasone, has been shown to improve transduction efficiency [187, 188]. While LV vectors trigger a less intense IFN-alpha response from plasmacytoid dendritic cells (pDCs) compared to HIV-1, pDCs are believed to play a critical role in the T1 IFN response to LVs. Toll-like receptors TLR7 and TLR9, which detect single-stranded RNA in endosomes, are instrumental in inducing T1 IFN production [189, 190].

VSV-G pseudotyped LV vectors may also contain tubulovesicular structures with DNA fragments, enhancing TLR9 signaling. The downstream signaling of TLR7 and TLR9 is partially regulated by the mammalian target of rapamycin (mTOR) pathway. Research by Brown et al.

Table 5 Challenges in AMD gene therapy: host immune responses to viral vectors and transgene products

Immune Response	Challenge	Mechanism	Potential Impact on Gene Therapy	References
Pre-existing Immunity	Neutralization by existing antibodies	Antibodies from prior infections may neutralize vectors before gene delivery	May prevent the vector from delivering the therapeutic gene	[181]
Innate Immune Response	Inflammation triggered by vector recognition	Recognition of viral elements by the immune system incites inflammation	Could diminish the efficacy of the therapy	[181]
Adaptive T Cell Response	Activation of cytotoxic T cells	Dendritic cells present viral antigens to T cells, activating CTLs that destroy vector-infected cells	May lead to the eradication of therapeutic gene-expressing cells	[181]
High-dose Vector Toxicity	Complement system activation and broader toxicities	High-dose vectors may trigger immune segments like the complement system, leading to cell surface vulnerability and other toxicities	Could cause damage across various organs or the central nervous system	[181]
LV Vector Phagocytosis	Decreased distribution to target cells	Phagocytosis by immune cells reduces the availability of vectors to target cells	May limit the effectiveness of hepatic gene transfer	[186]
Type I Interferon Production	Reduced transduction efficiency	Production of T1 IFN in response to LV vectors can decrease transduction efficiency	Pharmacological inhibition of IFN production may be necessary to improve outcomes	[187, 188]
TLR7 and TLR9 Signaling	Induction of T1 IFN	Toll-like receptors detect viral RNA, leading to T1 IFN production	Designing vectors to prevent transgene expression in pDCs may mitigate this response	[189–191]
cGAS-STING Pathway Activation	IFN-alpha response	Detection of viral DNA genomes by cGAS-STING pathway induces T1 IFN production	Understanding this pathway is crucial for designing vectors that avoid inducing strong IFN-alpha responses	[194, 195]

revealed that miR126, a microRNA essential for vascular endothelial cell function during angiogenesis, is uniquely expressed in pDCs and targets a negative regulator of mTOR, which is critical for generating pDCs and facilitating TLR-mediated innate responses to nucleic acids and LV vectors. These insights can be used to design vectors that avoid transgene expression in pDCs by incorporating target sequences within the transcript [191–193]. However, merely inhibiting TLR7 or TLR9 signaling is insufficient to prevent LV vectors from inducing IFN-alpha responses, likely due to the detection of viral DNA genomes by the cGAS-STING pathway, a cytoplasmic mechanism involved in recognizing DNA through cyclic GMP-AMP synthase (cGAS) and its downstream adaptor STING. Activation of STING in the endoplasmic reticulum (ER) triggers IRF3 phosphorylation via TBK1, leading to the production of type I IFN [194, 195]. (Table 5)

A comparative examination of preclinical and clinical data underscores key differences in immunogenicity and the ensuing risk of inflammation by divergent delivery modalities and vector systems utilized in gene therapy for AMD. These sorts of parameters, i.e., subretinal vs. intravitreal delivery and AAV vs. lentiviral vectors, play a crucial role in establishing therapeutic windows and safety profiles, especially with respect to the immune environment relevant to aging in AMD.

Subretinal vs. Intravitreal Subretinal injection is generally at less risk of anterior segment inflammation but carries a risk of localized retinal atrophy and pigmentary changes, particularly with higher vector doses. Clinical experience in the RGX-314 trial with AAV8 revealed a favorable safety profile, with no clinically significant immune responses beyond those associated with the vitrectomy procedure itself [196–198].

Nonetheless, one incident of profound vision loss caused by pigmentary changes was noted at the high dose level, and asymptomatic peripheral pigmentary effects were observed in several patients who had received moderate to high doses. Although this method enables very precise delivery to the subretinal space, it presents technical difficulty to elderly AMD patients with potentially thin or atrophic retinas, and therefore a higher risk of damage or procedural complications. In contrast, intravitreal injection directly exposes vectors to the ocular immune system, which more frequently leads to an increased incidence of anterior chamber inflammation such as anterior uveitis and vitritis. This was observed in ADVM-022 (AAV2.7m8) clinical trials, where phase 1/2 data indicated up to a 40% incidence of anterior uveitis at the higher doses. Fortunately, most inflammatory events were mild to moderate in severity and responded well to topical corticosteroids, and there were no instances of vision-threatening inflammation. By comparison, older patients with age-related macular degeneration frequently have elevated baseline ocular inflammation and pre-existing anti-AAV antibodies, which can worsen the immune response and reduce therapeutic effect [196–198].

Based on the comparison of vector types, AAV vectors, by different serotypes, will have a mild and transient inflammatory profile with low proinflammatory cytokine induction compared with lentiviral systems. RGX-314

subretinally delivered via AAV8 caused low inflammation and no unexpected immune responses, which underlines the preference of AAV for application in AMD indications. ADVM-022 administered intravitreally too had managed inflammation, which was largely steroid-responsive. Nonetheless, the elevated seroprevalence of neutralizing antibodies in senior populations, alongside immune dysregulation linked to aging, may enhance host responses to AAV capsids, especially when administered at increased dosages [198–201].

Lentiviral vectors, although efficient in specific scenarios, are linked to an increased likelihood of activating the innate immune system. Evidence indicates that they can provoke self-limiting inflammatory reactions, characterized by a rise in interleukin-6 (IL-6) levels. Clinical testing of RetinoStat, a subretinally delivered lentiviral vector, demonstrated moderate to severe inflammation and other serious adverse effects in high-dose cohorts, such as uveitis and chorioretinopathy. One instance of retinal hole was observed. Because of their decreased transduction efficiency in post-mitotic retinal cells and increased risk of inflammatory complications, lentiviral vectors may be less ideal for AMD gene therapy applications. In conclusion, both vector choice and delivery route play important roles in determining inflammation risk and immune activation in AMD gene therapy. Subretinal AAV delivery is less risky from an inflammatory standpoint but is invasive surgically, whereas intravitreal procedures, while less invasive, are associated with higher risks of immunogenicity, particularly in the aging eye. AAV vectors remain the most promising vector due to their favorable balance of safety and efficacy, although ongoing strategies to avoid immune responses, capsid engineering, immunosuppression, and codon optimization, are essential to ultimate long-term success with AMD treatment [202–204].

Regarding immunogenicity mitigation strategies, AMD poses specific immunological hurdles to gene therapy in the form of immune dysregulation, augmented ocular inflammation, and high prevalence of anti-vector antibodies in elderly patients. Management of these elements necessitates the design of individualized strategies to provide both safety and effectiveness. Immunosuppression, particularly with topical corticosteroids dexamethasone and prednisolone, is required for the management of inflammation after intravitreal AAV administration. Periocular or systemic steroids can be utilized in severe circumstances but must be used carefully in elderly patients due to systemic complications. Moreover, vector engineering with transduced AAV capsids (e.g., AAV7m8, AAV8) and capsid shielding strategies has the potential to reduce immune recognition, which is critical in AMD patients with high anti-AAV antibody titers. Tissue-specific promoters targeting RPE or photoreceptors help to minimize off-target transgene expression and antigen presentation, reducing immune activation in the inflamed retina. Dose optimization also provides a balance between therapeutic efficacy and immune response, especially in eyes with compromised retinal barriers [198, 201].

Pre-screening for anti-AAV antibodies helps identify high-risk patients and guide immunosuppressive protocols. Cumulatively, these strategies ranging from immunosuppression, vector and promoter design, dosing, and screening are an integrated platform for safe and effective gene therapy in AMD.

Long-term durability: sustained gene expression and therapeutic effects in AMD patients

Research shows that genetic material from rAAV vectors can remain stable for extended periods within cells, underscoring its potential for long-lasting retinal gene therapy. In rodent models, rAAV episomes have demonstrated durability for approximately 18 months, which is consistent with their shorter lifespans [48]. However, in canine models, the effects persist for up to 9.4 years, a duration comparable to their late life stages [55]. Despite the prolonged presence of rAAV episomes, sustained expression of the RPE65 enzyme is not guaranteed, as other, yet-to-be-identified factors may influence gene expression.

Longevity of expression is affected by the choice of promoters and proteins used in RPE65 gene studies [65, 66]. For example, the cytomegalovirus (CMV) promoter may become silenced over time due to methylation, leading to reduced transgene expression [35, 67]. Future studies must address how these findings translate to human gene therapy, particularly regarding the factors affecting episomal stability and transgene expression. Although there is currently no evidence suggesting that such episomal genetic material would not persist in humans, it is equally important to investigate the potential for rare integration events, especially in cases of double-stranded DNA breaks.

RPE cells, established early in development and with minimal proliferation throughout life, enable continuous transgene expression without the need for genomic integration [33]. Under certain conditions, RPE cells can replicate and restore function after receiving a functional RPE65 gene, suggesting that successful gene incorporation could lead to lifelong cell functionality. This has critical implications for the durability of retinal gene therapies targeting RPE cells, such as those developed for voretigene neparvovec (VN).

Recent advances in rAAV vector design—aimed at achieving high expression levels with fewer viral particles—are addressing some of the challenges associated with gene therapy, including reducing inflammation and

toxicity risks [68]. However, various factors, including capsid composition and vector production processes, can influence the durability of the treatment's effects [33].

The timing of gene therapy in relation to the disease's progression is another critical factor for long-term success. The number and viability of retinal cells generally decline over time, impacting the effectiveness of the treatment. Although optimal results are typically observed in younger animals [52, 55]. Treatments at later disease stages can still be effective if enough viable photoreceptors remain [47, 69]. Clinical data indicate that even older patients with an adequate number of outer retinal cells at the time of treatment have benefited from gene therapy, with no significant age-related differences observed in outcomes one year after treatment [22, 38, 70].

Surgical technique plays a pivotal role in ensuring successful outcomes. Factors such as injection method and site are critical for maximizing product delivery while minimizing risks [71]. Advances in vector capsids that allow for broader transduction with fewer viral particles have shown great promise [72–74]. The surgical approach, combined with the optimal viral dosage and expression levels, significantly impacts the longevity of the therapeutic response.

Although multiple injections could expand treatment coverage, they also increase the risks of complications. Higher injection volumes, for instance, are associated with increased risks of retinal detachment and immune responses [36, 75]. Capsids engineered for wider distribution and promoters that drive high-level expression specific to target cells may help mitigate these risks [73, 74]. Ongoing research is focused on improving treatment coverage while minimizing immune reactions, with the goal of enhancing patient outcomes. A deeper understanding of immune responses in ocular gene therapy will be essential for developing strategies that maximize therapeutic benefits while minimizing risks [76].

Finally, gene augmentation therapies such as VN rely on the availability of viable retinal cells [4, 20, 69, 77]. Studies in mouse models suggest that early delivery of functional RPE65 protein could have indirect benefits for surrounding cells, supporting their health and prolonging their survival [69, 77].

The variability in expression longevity is influenced by the choice of promoters and proteins used in RPE65 animal studies [65, 66]. For instance, the CMV promoter might be silenced over time due to methylation, leading to reduced expression of the AAV transgene [35, 67, 188]. Future studies should focus on how these findings translate to human gene therapy, particularly the factors influencing episomal stability and transgene expression. Currently, there is no evidence suggesting that such episomal genetic material wouldn't persist in humans, nor are there studies ruling out the possibility of rare integration events in the case of double-stranded DNA breaks.

RPE cells, which are established early in development and proliferate minimally, allow for the continuous expression of the transgene without genomic integration [33]. These cells can replicate under certain conditions and can restore balance after receiving a functional RPE65 gene, which suggests that successful gene incorporation could lead to lifelong cell functionality. This has significant implications for the expected duration of retinal gene therapies targeting RPE cells, such as VN.

The development of rAAV vectors that achieve high expression levels with fewer particles is addressing the challenges of gene therapy's long-term effects, reducing inflammation and toxicity risks [68]. Nonetheless, various factors such as capsid composition and the vector production process can affect the treatment's durability [33].

The timing of the gene therapy relative to the disease's progression is also crucial for long-term success, depending on the number and viability of retinal cells, which typically decline over time. Although optimal results have been observed in younger animals [52, 55], treatments in later disease stages can still be effective if enough viable photoreceptors remain [47, 69]. Clinical data show that older patients with a sufficient number of outer retinal cells at the time of treatment have benefited from gene therapy [22, 38], with no significant age-related differences observed in outcomes one year after treatment [70].

Surgical details such as injection technique and site are pivotal for successful outcomes and should be fine-tuned to maximize product delivery while minimizing risks [71]. Advances in vector capsids that allow for broader spread and transduction with fewer particles are significant [72–74]. The surgical approach also influences the dosage and expression levels, which are key to determining the optimal viral dose and response longevity.

While multiple injections could treat larger retinal areas, they come with increased risks. High injection volumes are associated with an increased chance of retinal detachment and immune responses [36, 75]. Capsids designed for wider spread and promoters enabling high-level expression specific to target cells could help mitigate these risks [73, 74]. Ongoing research aims to enhance treatment coverage with minimal immune reaction and improved patient outcomes. A deeper understanding of immune responses to ocular gene therapy will help to identify strategies to maximize therapeutic benefits while minimizing risks [76].

Gene augmentation treatments such as VN depend on the availability of viable retinal cells [4, 20]. Studies in mouse models suggest that providing a functional RPE65

protein early on could indirectly benefit surrounding cells, enhancing their health and longevity [69, 77].

Effective targeting: vector selection and targeted engineering

Regulating the precise delivery of gene therapy to specific tissues or cells presents a complex challenge, particularly when systemic delivery is obstructed by barriers such as the blood-retina barrier (BRB), which hinders access to the retina [205]. It is also critical to ensure effective transduction of target diseased cells. Utilizing specialized vectors or cell-specific promoters can enhance precision, while direct delivery to the affected tissue can maximize therapeutic impact [206–208].

AAVs have inherent tissue tropism, which makes certain AAV serotypes including AAV2, AAV4, AAV5, and AAV8—particularly well-suited for retinal gene therapy [209, 210]. Tailoring vector capsids through recombinant engineering can refine tissue targeting, allowing more controlled interactions with vector components. This specificity is further enhanced using cell-specific promoters [207, 211]. For example, AAV1, AAV4, AAV6, and the engineered AAV2-7m8 vector can effectively target RPE cells, while AAV2, AAV5, AAV7, AAV8, AAV9, and engineered variants such as AAV2-7m8 and AAV8BP2 are used for photoreceptor and Müller glial cell transduction [208, 212–216]. A recent initiative involving the engineering of 230 AAVs, each with a synthetic promoter for specific cell types, illustrates the ongoing advancements in this area [211].

Once vector design is finalized, the challenge becomes selecting the most effective delivery method. While gene therapies can be administered ocularly or systemically, localized delivery methods—such as subretinal or intravitreal injections are generally preferred for their proximity to the target area [208]. Systemic delivery, though convenient, can cause off-target effects, reduce bioavailability at the target site, and heighten immunogenicity risks. In contrast, ocular delivery confines the therapy to the intended tissue, reducing immune reactions. However, invasive ocular methods come with their own risks, including potential complications that require expert surgical precision [217]. Conversely, non-invasive methods offer safer delivery with fewer complications but typically result in lower bioavailability [218].

Non-invasive options include topical applications (e.g., eye drops), iontophoresis, ultrasonics, transdermal systems, and contact lenses. These methods avoid surgical risks but may offer limited bioavailability. For instance, topical administration, the most common non-invasive method, allows for self-administration but faces challenges with low bioavailability (5% or less) due to the ocular barrier [218, 219]. Iontophoresis, which uses mild electrical currents to enhance drug penetration, and ultrasonic devices or transdermal patches can improve penetration and controlled release [219–221]. Contact lenses soaked in drug solutions provide another alternative, offering sustained release and better compliance, though they are limited by drug solubility [222]. Choosing the optimal delivery method is essential for ensuring efficient transduction and minimizing immune responses.

Invasive techniques, such as ocular implants, subtenon, and subconjunctival injections, are less common but provide targeted delivery. Subtenon injections, effective for conditions like diabetic macular edema and choroidal detachments, may elevate intraocular pressure [223, 224]. Subconjunctival injections offer better bioavailability than topical methods but may be absorbed systemically. Ocular implants, both biodegradable and non-biodegradable, are employed for sustained drug release and controlled delivery [225].

Among invasive methods, subretinal administration which introduces the therapy between the photoreceptors and RPE layer requires less vector to achieve efficacy compared to intravitreal injections [126]. However, this approach carries risks of exacerbating retinal damage, potentially leading to detachment, hemorrhage, or pigmentation changes, with the delicate fovea being particularly vulnerable [226–228]. Despite these risks, subretinal injections using AAV8, AAV2, or AAV5 have not been linked to severe inflammation, making this method viable [209, 229]. Corticosteroids are often used to mitigate inflammation and immune reactions following the procedure, and advances in vector capsid engineering and robotic surgical assistance may help reduce complications [229].

Intravitreal injections, which target the inner retina, are less invasive and commonly used in therapies for AMD and diabetic retinopathy [226, 230]. While this method is effective, it poses risks, including inflammation, infection, increased pressure, detachment, hemorrhage, and cataracts, along with patient compliance issues due to the need for repeated injections [230]. Most viral vectors face challenges crossing the inner limiting membrane, limiting their ability to reach photoreceptors and RPE [226]. However, recombinant vectors such as AAV2. GL and AAV2. NN have shown improved delivery [208, 231]. Intravitreal injections can also trigger systemic side effects and are more immunogenic than subretinal methods [209]. Notably, Adverum Biotechnologies halted the development of an intravitreal AAV-based gene therapy due to toxicity concerns [232]. (Table 6).

Suprachoroidal injections, administered between the choroid and sclera, offer better bioavailability than intravitreal methods without requiring vitreoretinal surgery [233]. However, even skilled surgeons face potential complications such as hemorrhage, infection, choroidal tears,

Table 6 Challenges in AMD therapy: vector selection and effective engineering

Challenge	Details	Implications	Strategies for Improvement	References
Blood-Retina Barrier	Obstructs systemic gene therapies from accessing the retina	Requires precise delivery methods to reach target cells	Use of cell-specific vectors/promoters and direct tissue delivery	[205, 206, 208]
Tissue Preferences of AAVs	Certain AAV serotypes are preferred for retinal gene therapy	Need for serotypes that effectively transduce target cells	Tailoring vector capsids and using specific promoters for cell types	[209–211]
Targeting Specific Cells	Different AAV serotypes and engineered vectors target various retinal cells	Ensures therapy reaches diseased cells	Strategic use of AAV serotypes and engineered vectors for cell-specific targeting	[211–216]
Delivery Method	Localized routes preferred for proximity to target area	Systemic delivery may cause off-target effects and immunogenicity	Ocular delivery methods to confine effects to intended tissue	[217, 218]
Non-invasive Delivery Options	Topical applications, iontophoresis, ultrasonics, transdermal systems, contact lenses	Safer but may offer less bioavailability	Balance between safety and bioavailability to be considered	[218–222]
Invasive Delivery Techniques	Ocular implants, subtenon, subconjunctival injections	Less common due to risks and complications	Use of less invasive methods, when possible, to minimize risks	[223–225]
Subretinal Administration	Introduces drugs between photoreceptor cells and RPE layer	Risks damage to compromised retina but requires less vector	Advances in surgical techniques and vector engineering to reduce risks	[130, 226–229, 234]
Intravitreal Injections	Target the inner retina	Less invasive but pose risks of inflammation, infection, and other complications	Novel recombinant vectors for improved delivery and reduced risks	[226, 230, 231]
Suprachoroidal Injections	Placed between choroid and sclera	Better bioavailability without vitreoretinal surgery but with potential complications	Skilled surgical execution to maximize success and minimize risks	[233]

and retinal detachment [233]. Determining the most appropriate delivery method is essential for maximizing gene therapy's success while minimizing patient risk.

Strategies to overcome challenges in AMD gene therapy

Immune modulation strategies: immunosuppression and immune tolerance induction

Many people develop an immune response to the capsid of natural AAV, with antibodies detectable from individuals as young as two years old [235–237]. This immune reaction arises due to the similarity in amino acid sequences across various AAV serotypes, both natural and synthetic, leading to antibodies capable of recognizing and binding to these viral capsids [238]. While preexisting neutralizing antibodies (NAbs) do not interfere with gene therapies targeted at the eye or directly injected into solid tissues, they pose a significant challenge for treatments requiring systemic circulation, such as those targeting the liver.

The impact of NAbs was notably demonstrated in a pioneering clinical trial aimed at the liver using AAV vectors. In this trial, two participants received the same dosage, but their outcomes varied based on pre-treatment NAb levels [239]. The participant with lower NAb levels exhibited a 12% normal expression of Factor IX (FIX), whereas the one with higher NAbs showed no transgene expression at all. Animal studies further confirmed that NAb levels of 1:5 or higher could block liver transduction following intravenous delivery [240, 241]. As a result, clinical trials now screen for NAbs and exclude individuals with high levels to improve the likelihood of treatment success [242].

Currently, individuals with detectable NAbs are often ineligible for gene therapy trials, as their presence can interfere with the effectiveness of the treatment. Furthermore, after the initial administration, the body may produce high levels of NAbs, which can prevent subsequent treatments with the same AAV vector [243]. This limitation highlights the urgent need for strategies to overcome NAbs, enabling effective treatment and allowing for potential re-administration of AAV-based therapies in all patients, regardless of pre-existing or post-treatment antibody levels.

Vector engineering: enhancing viral vector tropism and transduction efficiency

To enhance the expression of recombinant AAV (rAAV) vectors upon transduction, several strategies can be employed. These include altering AAV inverted terminal repeats (ITRs) to allow transgene expression without the need for second-strand DNA synthesis, optimizing the promoter to increase transcription, refining codon usage within the transgene to boost mRNA production and translation, and improving the delivery of larger transgenes [244].

Altering AAV ITRs can significantly enhance the rate of AAV transduction, which is often slowed by the need for double-stranded DNA (dsDNA) synthesis from the single-stranded AAV genome before mRNA transcription can begin. By modifying one of the wild-type ITRs, this crucial molecular step can be bypassed. The mutation

renders the ITR an unsuitable substrate for Rep68 and Rep78 proteins, preventing terminal resolution during replication. This modification leads to the formation of self-complementary AAV (scAAV) replication intermediates, which package both strands of DNA into the viral particle, rather than a single strand as seen in wild-type AAV.

Upon delivery to the nucleus, the two complementary DNA strands joined by the altered ITR immediately anneal to form dsDNA, allowing for rapid transcription without the need for further synthesis. This results in significantly faster and more robust expression of proteins, such as GFP or Factor IX (FIX), when encoded by scAAV vectors compared to traditional single-stranded AAV vectors.

By 2020, self-complementary AAV8 (scAAV8) had achieved a decade of clinical trial success in treating haemophilia B, and scAAV vectors became integral to Zolgensma, the FDA-approved treatment for spinal muscular atrophy. While ITR engineering enhances AAV transduction, scAAV vectors are limited in their ability to carry large transgene cassettes. To address this, in silico methods have been employed to develop promoters that facilitate liver-specific transgene expression via AAV. A comprehensive screening process identified multiple hepatocyte-specific cis-acting regulatory modules that, when combined with liver-specific promoters in rAAV cassettes, significantly boosted transgene expression in mice. This approach also identified cardiac-specific regulatory modules, offering the potential to design vectors for more targeted transduction in various tissues.

Biotechnology firms are now focusing on promoter optimization for gene therapy, suggesting that future rAAV vectors could be modular, with each component optimized for AAV expression and validated through clinical testing. One essential aspect of AAV transgene optimization involves refining the codons of therapeutic transgenes, which are often based on natural gene sequences and may not be optimized for enhanced transduction. Traditionally, codon optimization relies on the cDNA of the host species to match codon usage bias to the concentrations of individual tRNAs. However, because tRNA concentrations vary between tissues and cell types, a recent study focused on tissue- and cell-type-specific codon usage bias for liver-targeted gene therapy. In this study, rAAV vectors with codon-optimized sequences demonstrated higher expression levels of FVIII in human hepatocyte cell lines and mouse liver compared to wild-type sequences.

Codon optimization has since become a standard practice in developing rAAV vectors for clinical use, with vendors offering optimized codon sequences for efficient expression. However, caution is necessary, as different vendors' optimizations for the same transgene have produced varying expression levels when tested in rAAV vectors. Optimizing AAV vector packaging is also critical for delivering large transgenes, such as those needed to treat conditions like Duchenne muscular dystrophy, haemophilia A, and Stargardt disease. Although truncated versions of these transgenes have shown some success in clinical trials, alternative strategies are being explored to transport larger transgenes using AAV vectors in animal models [245–250].

The first method for expanding AAV vector capacity leverages the AAV genome's ability to form concatemers through homologous recombination of ITR sequences after delivery. This allows for the division of large transgene cassettes across multiple vectors, which then recombine inside the cell nucleus to reconstruct the full transgene. This technique has shown success in animal studies, where separate AAV vectors were delivered, leading to the production of functional dystrophin.

The second technique involves randomly packaging truncated transgene fragments into various AAV virions. Once inside the cell, these fragments undergo homologous recombination or annealing of complementary regions, leading to the formation of a complete transgene. Adding specific overlapping fragments to each AAV vector can further encourage recombination and improve the efficiency of this approach.

However, both methods have certain limitations. Concatemerization may result in non-functional recombinant products and using truncated overlapping genetic fragments risks introducing ITR structures into the transgene. To address these issues, a third strategy—the hybrid dual-vector approach—has been developed. This method combines an overlapping region with intron splice sites within the split transgenes. It relies on the concatemerization of AAV genomes to recombine separate AAV vector genomes, which are intentionally split into left and right halves containing 5′ and 3′ splicing elements. Once recombined, splicing ensures the production of the correct transgene protein. Research has demonstrated that inserting a highly recombinogenic sequence (e.g., 872 bp alkaline phosphatase) into AAV vectors with intron splice sites significantly increases homologous recombination in animal models, potentially improving the expression of functional proteins.

A fourth tactic involves cross packaging an AAV genome into the capsids of other parvoviruses. In vitro experiments have shown efficient transduction when AAV genomes were packaged into bocavirus parvovirus and parvovirus B19 capsids, creating chimeric vectors. Another strategy is intein-mediated protein trans-splicing, which allows for the design of split AAV vectors capable of packaging larger genomes. This method, akin to intron-mediated RNA splicing, joins two polypeptides through trans-splicing. By delivering multiple AAV

vectors, each carrying a fragment of the target protein flanked by short split inteins, a full-length protein can be formed in the target cells. This technique has successfully delivered proteins such as dystrophin, FVIII, CFTR, CRISPR–Cas9, ABCA4, and CEP290 in animal models.

While these methods expand the gene-size capacity of AAV vectors, dual-vector strategies have been less efficient at producing therapeutic proteins compared to single-vector methods. They also exhibit variable success in animals and carry the risk of expressing undesired products. Moving forward, these strategies may be more suitable for dividing cells or for transgene cassettes requiring temporary expression, such as those encoding CRISPR–Cas9, where temporary expression could minimize off-target effects from prolonged transgene expression. Additionally, AAV capsids can be tailored through techniques such as rational design, directed evolution, and the use of computationally designed ancestral capsids to improve vector performance [251–256].

Combination therapies: integrating gene therapy with Pharmacological interventions for synergistic effects

Viral vectors are the predominant method for systemic gene therapy delivery, used in nearly 70% of gene therapy clinical trials. Among these, adenoviral vectors (Ads) account for about 25%, primarily due to their exceptional in vivo transduction capabilities, robust transgene expression, large DNA carrying capacity, high stability, minimal pathogenicity in vivo, and their ability to infect both dividing and non-dividing cells [257].

The fundamental structure of Ads consists of an icosahedral capsid composed of hexon proteins, with a penton complex anchored at the vertices. This complex includes the penton base and projecting fiber proteins. Human adenoviruses from subgroup B (species 3, 7, 16, 21, 50, 11, 14, 34, 35) primarily attach to cells via the CD46 receptor, a complement regulator present on all human cells except red blood cells. In contrast, Ads from subgroups A, D, E, and F use the coxsackie and adenovirus receptor (CAR) to initiate viral entry. The D1 domain of CAR binds to the carboxyterminal knob domain of serotype 5 fibers, facilitating viral internalization via Rv3 and Rv5 integrins through the arginine-glycine-aspartic acid (RGD) motif in the adenoviral penton base. Once internalized, the virus enters a clathrin-coated endosome, where it is released into the cytoplasm following endosomal acidification, allowing it to move to the nuclear pore for genome replication.

When administered systemically, adenoviral vectors predominantly accumulate in the liver, triggering an acute phase reaction characterized by surges in cytokines (e.g., TNF-α, IL-6) and chemokines (e.g., Mip-2, Ip-10), as well as the activation of leukocyte trafficking genes. This inflammatory response is primarily mediated by hepatic Kupffer cells (KCs), which shorten the lifespan of circulating viral particles and indirectly reduce transgene expression in other tissues. Depleting KCs using treatments such as clodronate, liposomes, or gadolinium chloride significantly enhances hepatocyte-specific Ad-mediated gene transfer, suggesting that KCs capture a substantial portion of the infectious viral particles. Initially, the CAR receptor was believed to be the primary factor driving the liver's viral uptake. However, efforts to prevent Ad sequestration in the liver by modifying or removing CAR- or integrin-binding motifs have largely been ineffective [257]. (Fig. 5)

Conclusions and future directions

Gene therapy has transitioned from a conceptual framework to a tangible clinical reality, as evidenced by more than 1,400 trials, showcasing its potential to cure retinal disease such as AMD. Therapies such as ADVM-022 and RGX-314 have the potential to provide permanent, one-time treatments that can greatly alleviate the treatment burden experienced by patients. Yet realizing the promise of these therapies depends on overcoming one of the field's most significant hurdles: affordability. Scalability and strategic production innovations must be developed to enable gene therapy to become more affordable and broadly available.

Scalable AAV production is among the most crucial areas of focus. Transitioning to suspension cell cultures and continuous bioprocessing at high yields can significantly reduce per-dose costs. Technologies such as TESSA (tetracycline-enabled self-silencing adenovirus) easily eliminate plasmid DNA impurities, therefore enabling purification steps and reducing batch-to-batch variation. In addition, standardized production processes are essential. Methods such as droplet digital PCR (ddPCR) allow exact quantification of vectors, and closed bioreactor systems reduce the potential for human error and increase reproducibility, critical factors to achieve regulatory compliance and reduce downstream expense.

The optimization of the payload also enhances cost-effectiveness. Technologies such as dual-vector systems and small promoters allow the delivery of larger genes in AAV payload constraints, which may reduce the need for repeat dosing. Such technology advancements have a direct impact on the cost-per-patient equation. For example, continuous manufacturing processes can increase AAV yields by 10–20 fold, and TESSA's plasmid-free platform has the potential to cut raw materials costs by 30–40%. These advances make therapies such as JNJ-81,201,887 or GT-005 more financially viable, providing a choice to current treatments such as Syfovre, which is priced at around $2,200 for every monthly injection.

Looking ahead, several trends are poised to reshape the landscape. Mutation-agnostic therapies, which target

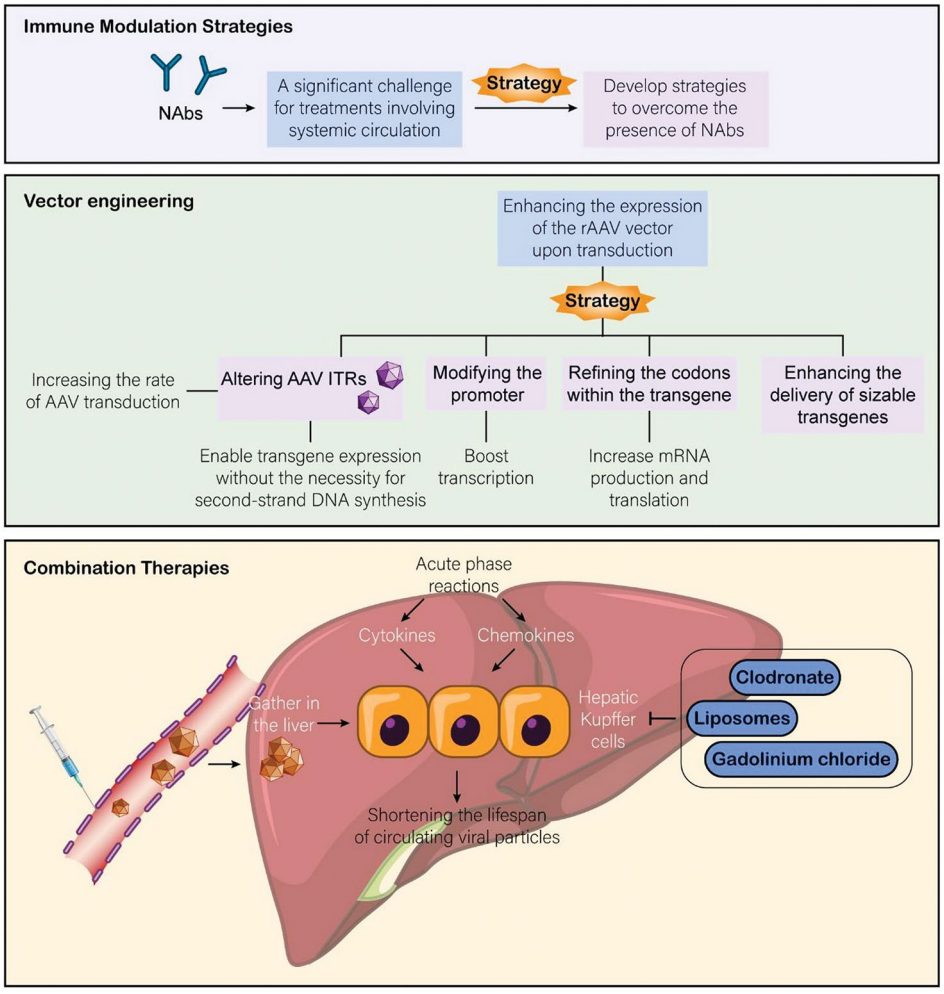


Fig. 5 Strategies to overcome gene therapy-related challenges. (i) Immune modulation strategies; (ii) Vector engineering; (iii) Combination therapies

modifier genes like CFH or employ optogenetics, could bypass the need for patient-specific solutions, broadening treatment eligibility. Next-generation vectors, such as AAV8-BP2 and lipid nanoparticles, will provide improved retinal targeting and enhanced transduction efficiency, allowing for lower therapeutic doses. In addition, the implementation of standardized worldwide manufacturing procedures, such as ISO-13,485 compliance for viral vector production, will enable harmonized regulatory strategies and cost-sharing on a global market basis.

Lastly, the future of retinal gene therapy relies not only on scientific breakthroughs but also on the redesigning of production processes. Through the combination of scalable AAV technologies, streamlined workflows, and mutation-independent strategies, the discipline can achieve a balance between therapeutic specificity and financial viability. Benchmark successes such as Luxturna represent the pinnacle of this twin success and as a blueprint for wider adoption in AMD. As the technologies mature, they can democratize gene therapy, bringing it into the mainstream of worldwide ophthalmic practice, saving the sight of millions of individuals across the globe.

Abbreviations

AAV	Adeno-associated Virus
ALK	001-Deuterated Vitamin A
AMD	Age-related Macular Degeneration
APO	Apolipoprotein
AREDS	Age-Related Eye Disease Study
ASM	Acid Sphingomyelinase
BCVA	Best-corrected Visual Acuity
BMI	Body Mass Index
BMP	Bis(monoacyl)glycerophosphate
CAR	Chimeric Antigen Receptor
CAR	Coxsackie and Adenovirus Receptor
CFH	Complement Factor H
cGAS	Cyclic GMP-AMP Synthase
CMV	Cytomegalovirus
CNV	Choroidal Neovascularisation
CRT	Central Retinal Thickness
CTL	Cytotoxic T Cell
DC	Dendritic Cell
DD	Destabilising Domain

DHFR	Dihydrofolate Reductase
dsDNA	Double-stranded Deoxyribonucleic Acid
ER	Endoplasmic Reticulum
FIX	Factor IX
GA	Geographic Atrophy
GFP	Green Fluorescent Protein
GWAS	Genome-wide Association Studies
HDL	High-density Lipoprotein
HE	Hybrid Exosome
hESC	Human Embryonic Stem Cell
HSC	Haematopoietic Stem Cell
IFN	Interferon
iPSC	Induced Pluripotent Stem Cell
ITR	Inverted Terminal Repeat
KC	Kupffer Cell
LCA	Leber's Congenital Amaurosis
LNP	Lipid Nanoparticle
LV	Lentiviral
LXR	Liver X Receptor
MAC	Membrane Attack Complex
MNV	Macular Neovascularisation
mTOR	Mammalian Target of Rapamycin
NAbs	Neutralising Antibodies
nAMD	Neovascular Age-related Macular Degeneration
OIR	Oxygen-induced Retinopathy
PCR	Polymerase Chain Reaction
PCV	Polypoidal Choroidal Vasculopathy
pDC	Plasmacytoid Dendritic Cell
rAAV	Recombinant Adeno-associated Virus
RBP4	Retinol Binding Protein 4
RGD	Arginine-Glycine-Aspartic Acid
ROS	Reactive Oxygen Species
RP	Retinitis Pigmentosa
RPC	Retinal Progenitor Cell
RPE	Retinal Pigment Epithelium
scAAV	Self-complementary Adeno-associated Virus Vector
sFlt-1	Soluble Human Vascular Endothelial Growth Factor Receptor 1
SSRI	Selective Serotonin Reuptake Inhibitor
TESSA	Tetracycline-enabled Self-silencing Adenovirus
TMP	Trimethoprim
VEGF	Vascular Endothelial Growth Factor
VN	Voretigene Neparvovec
VSV	Vesicular Stomatitis Virus

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KH, HYL, WMW, WT, SHD, RJR, NF, and APK conceptualized the study and contributed to investigation, writing the primary manuscript, and reviewing, writing the second draft and subsequent revisions of this manuscript; KH, NF, and APK were responsible for project administration and supervision, and contributed to proofreading, editing the first manuscript and subsequent revisions. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

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Competing interests
The authors declare no competing interests.

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References

1. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. Lancet. 2018;392(10153):1147–59.
2. de Jong EK, Geerlings MJ, den Hollander AI. Age-related macular degeneration. Genetics and genomics of eye disease. 2020:155–80.
3. Deng Y, Qiao L, Du M, Qu C, Wan L, Li J, Huang L. Age-related macular degeneration: epidemiology, genetics, pathophysiology, diagnosis, and targeted therapy. Genes Dis. 2022;9(1):62–79. https://doi.org/10.1016/j.gendis.2021.02.009.
4. Davis MD, Gangnon RE, Lee LY, Hubbard LD, Klein BE, Klein R, Ferris FL, Bressler SB, Milton RC. Age-Related eye disease study G. The Age-Related eye disease study severity scale for age-related macular degeneration: AREDS report 17. Arch Ophthalmol. 2005;123(11):1484–98. https://doi.org/10.1001/archopht.123.11.1484.
5. Ferris FL, Davis MD, Clemons TE, Lee LY, Chew EY, Lindblad AS, Milton RC, Bressler SB, Klein R. Age-Related eye disease study research G. A simplified severity scale for age-related macular degeneration: AREDS report 18. Arch Ophthalmol. 2005;123(11):1570–4. https://doi.org/10.1001/archopht.123.11.1570.
6. Liew G, Joachim N, Mitchell P, Burlutsky G, Wang JJ. Validating the AREDS simplified severity scale of Age-Related macular degeneration with 5- and 10-Year incident data in a Population-Based sample. Ophthalmology. 2016;123(9):1874–8. https://doi.org/10.1016/j.ophtha.2016.05.043.
7. Ashique S, Kumar S, Hussain A, Farid A, Mishra N, Garg A. Age-associated macular degeneration: epidemiologic features, complications, and potential therapeutic approaches. Targeting angiogenesis, inflammation, and oxidative stress in chronic diseases. Elsevier; 2024. pp. 381–429.
8. Wong TY, Chakravarthy U, Klein R, Mitchell P, Zlateva G, Buggage R, Fahrbach K, Probst C, Sledge I. The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. Ophthalmology. 2008;115(1):116–26. https://doi.org/10.1016/j.ophtha.2007.03.008.
9. Hernandez-Zimbron LF, Zamora-Alvarado R, Ochoa-De la Paz L, Velez-Montoya R, Zenteno E, Gulias-Canizo R, Quiroz-Mercado H, Gonzalez-Salinas R. Age-Related macular degeneration: new paradigms for treatment and management of AMD. Oxid Med Cell Longev. 2018;2018:8374647. https://doi.org/10.1155/2018/8374647.
10. Kumbhar P, Kolekar K, Vishwas S, Shetti P, Kumbar V, Andreoli Pinto TJ, Paiva-Santos AC, Veiga F, Gupta G, Singh SK, Dua K, Disouza J, Patravale V. Treatment avenues for age-related macular degeneration: breakthroughs and bottlenecks. Ageing Res Rev. 2024;98:102322DOI. https://doi.org/10.1016/j.arr.2024.102322.

11. Fairbanks AM, Husain D, editors. Controversies and disparities in the management of Age-Related macular degeneration. Seminars in ophthalmology. Taylor & Francis; 2023.
12. Heier JS, Lad EM, Holz FG, Rosenfeld PJ, Guymer RH, Boyer D, Grossi F, Baumal CR, Korobelnik JF, Slakter JS, Waheed NK, Metlapally R, Pearce I, Steinle N, Francone AA, Hu A, Lally DR, Deschatelets P, Francois C, Bliss C, Staurenghi G, Mones J, Singh RP, Ribeiro R, Wykoff CC. Oaks, investigators Ds. Pegcetacoplan for the treatment of geographic atrophy secondary to age-related macular degeneration (OAKS and DERBY): two multicentre, randomised, double-masked, sham-controlled, phase 3 trials. Lancet. 2023;402(10411):1434–48. https://doi.org/10.1016/S0140-6736(23)01520-9.
13. Nadeem A, Malik IA, Shariq F, Afridi EK, Taha M, Raufi N, Naveed AK, Iqbal J, Habte A. Advancements in the treatment of geographic atrophy: focus on Pegcetacoplan in age-related macular degeneration. Ann Med Surg (Lond). 2023;85(12):6067–77. https://doi.org/10.1097/MS9.0000000000001466.
14. Shakeel L, Khan A, Akilimali A. Izervay (avacincaptad pegol): paving the way for vision preservation in geographic atrophy. Ann Med Surg (Lond). 2024;86(5):2413–6. https://doi.org/10.1097/MS9.0000000000002021.
15. Kang C. Avacincaptad Pegol: first approval. Drugs. 2023;83(15):1447–53. https://doi.org/10.1007/s40265-023-01948-8.
16. Singer MA, Awh CC, Sadda S, Freeman WR, Antoszyk AN, Wong P, Tuomi L. HORIZON: an open-label extension trial of Ranibizumab for choroidal neovascularization secondary to age-related macular degeneration. Ophthalmology. 2012;119(6):1175–83. https://doi.org/10.1016/j.ophtha.2011.12.016.
17. Comparison of Age-related Macular Degeneration Treatments Trials, Research G, Maguire MG, Martin DF, Ying GS, Jaffe GJ, Daniel E, Grunwald JE, Toth CA, Ferris FL 3rd, Fine SL. Five-Year outcomes with Anti-Vascular endothelial growth factor treatment of neovascular Age-Related macular degeneration: the comparison of Age-Related macular degeneration treatments trials. Ophthalmology. 2016;123(8):1751–61. https://doi.org/10.1016/j.ophtha.2016.03.045.
18. Holz FG, Tadayoni R, Beatty S, Berger A, Cereda MG, Cortez R, Hoyng CB, Hykin P, Staurenghi G, Heldner S, Bogumil T, Heah T, Sivaprasad S. Multi-country real-life experience of anti-vascular endothelial growth factor therapy for wet age-related macular degeneration. Br J Ophthalmol. 2015;99(2):220–6. https://doi.org/10.1136/bjophthalmol-2014-305327.
19. Falk MK, Kemp H, Sørensen TL. Four-year treatment results of neovascular age-related macular degeneration with Ranibizumab and causes for discontinuation of treatment. Am J Ophthalmol. 2013;155(1):89–95. e3.
20. Arevalo JF, Lasave AF, Wu L, Acon D, Berrocal MH, Diaz-Llopis M, Gallego-Pinazo R, Serrano MA, Alezzandrini AA, Rojas S, Maia M, Lujan S, Pan-American Collaborative Retina Study G. INTRAVITREAL BEVACIZUMAB FOR CHOROIDAL NEOVASCULARIZATION IN AGE-RELATED MACULAR DEGENERATION. 5-Year results of the Pan-American collaborative retina study group. Retina. 2016;36(5):859–67. https://doi.org/10.1097/IAE.0000000000000827.
21. Sengillo JD, Justus S, Tsai YT, Cabral T, Tsang SH, editors. Gene and cell-based therapies for inherited retinal disorders: an update. American journal of medical genetics part C: seminars in medical genetics. Wiley Online Library; 2016.
22. Moore NA, Bracha P, Hussain RM, Morral N, Ciulla TA. Gene therapy for age-related macular degeneration. Expert Opin Biol Ther. 2017;17(10):1235–44. https://doi.org/10.1080/14712598.2017.1356817.
23. Fritsche LG, Fariss RN, Stambolian D, Abecasis GR, Curcio CA, Swaroop A. Age-related macular degeneration: genetics and biology coming together. Annu Rev Genomics Hum Genet. 2014;15:151–71. https://doi.org/10.1146/annurev-genom-090413-025610.
24. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. Science. 2005;308(5720):385–9. https://doi.org/10.1126/science.1109557.
25. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005;308(5720):419–21. https://doi.org/10.1126/science.1110359.
26. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005;308(5720):421–4. https://doi.org/10.1126/science.1110189.
27. Sepp T, Khan JC, Thurlby DA, Shahid H, Clayton DG, Moore AT, Bird AC, Yates JR, Group GFiAS. Complement factor H variant Y402H is a major risk determinant for geographic atrophy and choroidal neovascularization in smokers and nonsmokers. Investig Ophthalmol Vis Sci. 2006;47(2):536–40.
28. Despriet DD, Klaver CC, Witteman JC, Bergen AA, Kardys I, de Maat MP, Boekhoorn SS, Vingerling JR, Hofman A, Oostra BA, Uitterlinden AG, Stijnen T, van Duijn CM, de Jong PT. Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. JAMA. 2006;296(3):301–9. https://doi.org/10.1001/jama.296.3.301.
29. Kaur I, Katta S, Reddy RK, Narayanan R, Mathai A, Majji AB, Chakrabarti S. The involvement of complement factor B and complement component C2 in an Indian cohort with age-related macular degeneration. Invest Ophthalmol Vis Sci. 2010;51(1):59–63. https://doi.org/10.1167/iovs.09-4135.
30. Armento A, Ueffing M, Clark SJ. The complement system in age-related macular degeneration. Cell Mol Life Sci. 2021;78(10):4487–505. https://doi.org/10.1007/s00018-021-03796-9.
31. Ennis S, Gibson J, Cree AJ, Collins A, Lotery AJ. Support for the involvement of complement factor I in age-related macular degeneration. Eur J Hum Genet. 2010;18(1):15–6. https://doi.org/10.1038/ejhg.2009.113.
32. Gliem M, Muller PL, Mangold E, Holz FG, Bolz HJ, Stohr H, Weber BH, Charbel Issa P. Sorsby fundus dystrophy: novel mutations, novel phenotypic characteristics, and treatment outcomes. Invest Ophthalmol Vis Sci. 2015;56(4):2664–76. https://doi.org/10.1167/iovs.14-15733.
33. Neale BM, Fagerness J, Reynolds R, Sobrin L, Parker M, Raychaudhuri S, Tan PL, Oh EC, Merriam JE, Souied E, Bernstein PS, Li B, Frederick JM, Zhang K, Brantley MA Jr., Lee AY, Zack DJ, Campochiaro B, Campochiaro P, Ripke S, Smith RT, Barile GR, Katsanis N, Allikmets R, Daly MJ, Seddon JM. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci U S A. 2010;107(16):7395–400. https://doi.org/10.1073/pnas.0912019107.
34. Ennis S, Jomary C, Mullins R, Cree A, Chen X, MacLeod A, Jones S, Collins A, Stone E, Lotery A. Association between the SERPING1 gene and age-related macular degeneration: a two-stage case–control study. Lancet. 2008;372(9652):1828–34.
35. Hu ML, Quinn J, Xue K. Interactions between Apolipoprotein E metabolism and retinal inflammation in age-related macular degeneration. Life. 2021;11(7):635.
36. Li CM, Clark ME, Chimento MF, Curcio CA. Apolipoprotein localization in isolated Drusen and retinal Apolipoprotein gene expression. Invest Ophthalmol Vis Sci. 2006;47(7):3119–28. https://doi.org/10.1167/iovs.05-1446.
37. Anderson DH, Ozaki S, Nealon M, Neitz J, Mullins RF, Hageman GS, Johnson LV. Local cellular sources of Apolipoprotein E in the human retina and retinal pigmented epithelium: implications for the process of Drusen formation. Am J Ophthalmol. 2001;131(6):767–81. https://doi.org/10.1016/s0002-9394(00)00961-2.
38. Eiriksdottir G, Aspelund T, Bjarnadottir K, Olafsdottir E, Launer LJ, Harris TB, Gudnason V. Apolipoprotein E genotype and Statins affect CRP levels through independent and different mechanisms: AGES-Reykjavik study. Atherosclerosis. 2006;186(1):222–4.
39. Strunnikova NV, Maminishkis A, Barb JJ, Wang F, Zhi C, Sergeev Y, Chen W, Edwards AO, Stambolian D, Abecasis G, Swaroop A, Munson PJ, Miller SS. Transcriptome analysis and molecular signature of human retinal pigment epithelium. Hum Mol Genet. 2010;19(12):2468–86. https://doi.org/10.1093/hmg/ddq129.
40. Fukuda R, Murakami T. Potential of Lipoprotein-Based nanoparticulate formulations for the treatment of eye diseases. Biol Pharm Bull. 2020;43(4):596–607. https://doi.org/10.1248/bpb.b19-00858.
41. [Available from: https://www.ebi.ac.uk/gwas/
42. Huang L, Zhang H, Cheng CY, Wen F, Tam PO, Zhao P, Chen H, Li Z, Chen L, Tai Z, Yamashiro K, Deng S, Zhu X, Chen W, Cai L, Lu F, Li Y, Cheung CM, Shi Y, Miyake M, Lin Y, Gong B, Liu X, Sim KS, Yang J, Mori K, Zhang X, Cackett PD, Tsujikawa M, Nishida K, Hao F, Ma S, Lin H, Cheng J, Fei P, Lai TY, Tang S, Laude A, Inoue S, Yeo IY, Sakurada Y, Zhou Y, Iijima H, Honda S, Lei C, Zhang L, Zheng H, Jiang D, Zhu X, Wong TY, Khor CC, Pang CP, Yoshimura N, Yang Z. A missense variant in FGD6 confers increased risk of polypoidal choroidal vasculopathy. Nat Genet. 2016;48(6):640–7. https://doi.org/10.1038/ng.3546.
43. Esparza-Gordillo J, Soria JM, Buil A, Almasy L, Blangero J, Fontcuberta J, Rodriguez de Cordoba S. Genetic and environmental factors influencing the human factor H plasma levels. Immunogenetics. 2004;56(2):77–82. https://doi.org/10.1007/s00251-004-0660-7.
44. DeAngelis MM, Ji F, Kim IK, Adams S, Capone A Jr., Ott J, Miller JW, Dryja TP. Cigarette smoking, CFH, APOE, ELOVL4, and risk of neovascular age-related macular degeneration. Arch Ophthalmol. 2007;125(1):49–54. https://doi.org/10.1001/archopht.125.1.49.

45. Sobrin L, Seddon JM. Nature and nurture-genes and environment-predict onset and progression of macular degeneration. Prog Retin Eye Res. 2014;40:1–15.
46. Seddon JM, George S, Rosner B, Klein ML. CFH gene variant, Y402H, and smoking, body mass index, environmental associations with advanced age-related macular degeneration. Hum Hered. 2006;61(3):157–65. https://doi.org/10.1159/000094141.
47. Wang JJ, Rochtchina E, Smith W, Klein R, Klein BE, Joshi T, Sivakumaran TA, Iyengar S, Mitchell P. Combined effects of complement factor H genotypes, fish consumption, and inflammatory markers on long-term risk for age-related macular degeneration in a cohort. Am J Epidemiol. 2009;169(5):633–41. https://doi.org/10.1093/aje/kwn358.
48. Ho L, van Leeuwen R, Witteman JC, van Duijn CM, Uitterlinden AG, Hofman A, de Jong PT, Vingerling JR, Klaver CC. Reducing the genetic risk of age-related macular degeneration with dietary antioxidants, zinc, and omega-3 fatty acids: the Rotterdam study. Arch Ophthalmol. 2011;129(6):758–66. https://doi.org/10.1001/archophthalmol.2011.141.
49. Wang JJ, Buitendijk GH, Rochtchina E, Lee KE, Klein BE, van Duijn CM, Flood VM, Meuer SM, Attia J, Myers C, Holliday EG, Tan AG, Smith WT, Iyengar SK, de Jong PT, Hofman A, Vingerling JR, Mitchell P, Klein R, Klaver CC. Genetic susceptibility, dietary antioxidants, and long-term incidence of age-related macular degeneration in two populations. Ophthalmology. 2014;121(3):667–75. https://doi.org/10.1016/j.ophtha.2013.10.017.
50. Seddon JM, Gensler G, Rosner B, C-reactive protein. ARMS2/HTRA1 gene variants are independently associated with risk of macular degeneration. Ophthalmology. 2010;117(8):1560–6. https://doi.org/10.1016/j.ophtha.2009.11.020.
51. Naj AC, Scott WK, Courtenay MD, Cade WH, Schwartz SG, Kovach JL, Agarwal A, Wang G, Haines JL, Pericak-Vance MA. Genetic factors in nonsmokers with age-related macular degeneration revealed through genome-wide gene-environment interaction analysis. Ann Hum Genet. 2013;77(3):215–31.
52. Schmidt S, Haines JL, Postel EA, Agarwal A, Kwan SY, Gilbert JR, Pericak-Vance MA, Scott WK. Joint effects of smoking history and APOE genotypes in age-related macular degeneration. Mol Vis. 2005;11:941–9.
53. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, Burdon KP, Hebbring SJ, Wen C, Gorski M, Kim IK, Cho D, Zack D, Souied E, Scholl HP, Bala E, Lee KE, Hunter DJ, Sardell RJ, Mitchell P, Merriam JE, Cipriani V, Hoffman JD, Schick T, Lechanteur YT, Guymer RH, Johnson MP, Jiang Y, Stanton CM, Buitendijk GH, Zhan X, Kwong AM, Boleda A, Brooks M, Gieser L, Ratnapriya R, Branham KE, Foerster JR, Heckenlively JR, Othman MI, Vote BJ, Liang HH, Souzeau E, McAllister IL, Isaacs T, Hall J, Lake S, Mackey DA, Constable IJ, Craig JE, Kitchner TE, Yang Z, Su Z, Luo H, Chen D, Ouyang H, Flagg K, Lin D, Mao G, Ferreyra H, Stark K, von Strachwitz CN, Wolf A, Brandl C, Rudolph G, Olden M, Morrison MA, Morgan DJ, Schu M, Ahn J, Silvestri G, Tsironi EE, Park KH, Farrer LA, Orlin A, Brucker A, Li M, Curcio CA, Mohand-Said S, Sahel JA, Audo I, Benchaboune M, Cree AJ, Rennie CA, Goverdhan SV, Grunin M, Hagbi-Levi S, Campochiaro P, Katsanis N, Holz FG, Blond F, Blanche H, Deleuze JF, Igo RP Jr., Truitt B, Peachey NS, Meuer SM, Myers CE, Moore EL, Klein R, Hauser MA, Postel EA, Courtenay MD, Schwartz SG, Kovach JL, Scott WK, Liew G, Tan AG, Gopinath B, Merriam JC, Smith RT, Khan JC, Shahid H, Moore AT, McGrath JA, Laux R, Brantley MA Jr., Agarwal A, Ersoy L, Caramoy A, Langmann T, Saksens NT, de Jong EK, Hoyng CB, Cain MS, Richardson AJ, Martin TM, Blangero J, Weeks DE, Dhillon B, van Duijn CM, Doheny KF, Romm J, Klaver CC, Hayward C, Gorin MB, Klein ML, Baird PN, den Hollander AI, Fauser S, Yates JR, Allikmets R, Wang JJ, Schaumberg DA, Klein BE, Hagstrom SA, Chowers I, Lotery AJ, Leveillard T, Zhang K, Brilliant MH, Hewitt AW, Swaroop A, Chew EY, Pericak-Vance MA, DeAngelis M, Stambolian D, Haines JL, Iyengar SK, Weber BH, Abecasis GR, Heid IM. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet. 2016;48(2):134–43.https://doi.org/10.1038/ng.3448
54. Colijn JM, den Hollander AI, Demirkan A, Cougnard-Gregoire A, Verzijden T, Kersten E, Meester-Smoor MA, Merle BMJ, Papageorgiou G, Ahmad S, Mulder MT, Costa MA, Benlian P, Bertelsen G, Bron AM, Claes B, Creuzot-Garcher C, Erke C, Fauser MG, Foster S, Hammond PJ, Hense CJ, Hoyng HW, Khawaja CB, Korobelnik AP, Piermarocchi JF, Segato T, Silva R, Souied EH, Williams KM, van Duijn CM, Delcourt C, Klaver CCW, European Eye Epidemiology C. Increased High-Density lipoprotein levels associated with Age-Related macular degeneration: evidence from the EYE-RISK and European eye epidemiology consortia. Ophthalmology. 2019;126(3):393–406. https://doi.org/10.1016/j.ophtha.2018.09.045. Consortium E-R.
55. Tan LX, Germer CJ, La Cunza N, Lakkaraju A. Complement activation, lipid metabolism, and mitochondrial injury: converging pathways in age-related macular degeneration. Redox Biol. 2020;37:101781DOI. https://doi.org/10.1016/j.redox.2020.101781.
56. Guziewicz KE, Sinha D, Gomez NM, Zorych K, Dutrow EV, Dhingra A, Mullins RF, Stone EM, Gamm DM, Boesze-Battaglia K, Aguirre GD. Bestrophinopathy: an RPE-photoreceptor interface disease. Prog Retin Eye Res. 2017;58:70–88. https://doi.org/10.1016/j.preteyeres.2017.01.005.
57. Storti F, Klee K, Todorova V, Steiner R, Othman A, van der Velde-Visser S, Samardzija M, Meneau I, Barben M, Karademir D, Pauzuolyte V, Boye SL, Blaser F, Ullmer C, Dunaief JL, Hornemann T, Rohrer L, den Hollander A, von Eckardstein A, Fingerle J, Maugeais C, Grimm C. Impaired ABCA1/ABCG1-mediated lipid efflux in the mouse retinal pigment epithelium (RPE) leads to retinal degeneration. Elife. 2019;8:e45100. https://doi.org/10.7554/eLife.45100.
58. Lakkaraju A, Finnemann SC, Rodriguez-Boulan E. The Lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. Proc Natl Acad Sci U S A. 2007;104(26):11026–31. https://doi.org/10.1073/pnas.0702504104.
59. Anderson DMG, Ablonczy Z, Koutalos Y, Hanneken AM, Spraggins JM, Calcutt MW, Crouch RK, Caprioli RM, Schey KL. Bis(monoacylglycero)phosphate lipids in the retinal pigment epithelium implicate lysosomal/endosomal dysfunction in a model of Stargardt disease and human retinas. Sci Rep. 2017;7(1):17352. https://doi.org/10.1038/s41598-017-17402-1.
60. Toops KA, Tan LX, Jiang Z, Radu RA, Lakkaraju A. Cholesterol-mediated activation of acid Sphingomyelinase disrupts autophagy in the retinal pigment epithelium. Mol Biol Cell. 2015;26(1):1–14. https://doi.org/10.1091/mbc.E14-05-1028.
61. Kaur G, Tan LX, Rathnasamy G, La Cunza N, Germer CJ, Toops KA, Fernandes M, Blenkinsop TA, Lakkaraju A. Aberrant early endosome biogenesis mediates complement activation in the retinal pigment epithelium in models of macular degeneration. Proc Natl Acad Sci U S A. 2018;115(36):9014–9. https://doi.org/10.1073/pnas.1805039115.
62. Tan LX, Toops KA, Lakkaraju A. Protective responses to sublytic complement in the retinal pigment epithelium. Proc Natl Acad Sci U S A. 2016;113(31):8789–94. https://doi.org/10.1073/pnas.1523061113.
63. Simón MV, Prado Spalm FH, Vera MS, Rotstein NP. Sphingolipids as emerging mediators in retina degeneration. Front Cell Neurosci. 2019;13:246.
64. Opreanu M, Tikhonenko M, Bozack S, Lydic TA, Reid GE, McSorley KM, Sochacki A, Perez GI, Esselman WJ, Kern T, Kolesnick R, Grant MB, Busik JV. The unconventional role of acid Sphingomyelinase in regulation of retinal microangiopathy in diabetic human and animal models. Diabetes. 2011;60(9):2370–8. https://doi.org/10.2337/db10-0550.
65. Chen H, Tran JA, Eckerd A, Huynh TP, Elliott MH, Brush RS, Mandal NA. Inhibition of de Novo ceramide biosynthesis by FTY720 protects rat retina from light-induced degeneration. J Lipid Res. 2013;54(6):1616–29. https://doi.org/10.1194/jlr.M035048.
66. Berry KAZ, Gordon WC, Murphy RC, Bazan NG. Spatial organization of lipids in the human retina and optic nerve by MALDI imaging mass spectrometry [S]. J Lipid Res. 2014;55(3):504–15.
67. Gordon WC, Berry KAZ, Murphy RC, Bazan NG. MALDI imaging mass spectrometry of lipids in age-related macular degeneration. Investig Ophthalmol Vis Sci. 2014;55(13):5234.
68. Pujol-Lereis LM, Liebisch G, Schick T, Lin Y, Grassmann F, Uchida K, Zipfel PF, Fauser S, Skerka C, Weber BHF. Evaluation of serum sphingolipids and the influence of genetic risk factors in age-related macular degeneration. PLoS ONE. 2018;13(8):e0200739. https://doi.org/10.1371/journal.pone.0200739.
69. Lakkaraju A, Umapathy A, Tan LX, Daniele L, Philp NJ, Boesze-Battaglia K, Williams DS. The cell biology of the retinal pigment epithelium. Prog Retin Eye Res. 2020;78:100846. https://doi.org/10.1016/j.preteyeres.2020.100846.
70. Yao J, Jia L, Khan N, Lin C, Mitter SK, Boulton ME, Dunaief JL, Klionsky DJ, Guan JL, Thompson DA, Zacks DN. Deletion of autophagy inducer RB1CC1 results in degeneration of the retinal pigment epithelium. Autophagy. 2015;11(6):939–53. https://doi.org/10.1080/15548627.2015.1041699.
71. Zhang Y, Cross SD, Stanton JB, Marmorstein AD, Le YZ, Marmorstein LY. Early AMD-like defects in the RPE and retinal degeneration in aged mice with RPE-specific deletion of Atg5 or Atg7. Mol Vis. 2017;23:228–41.
72. Mitter SK, Song C, Qi X, Mao H, Rao H, Akin D, Lewin A, Grant M, Dunn W Jr, Ding J. Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD. Autophagy. 2014;10(11):1989–2005.
73. Golestaneh N, Chu Y, Xiao Y-Y, Stoleru GL, Theos AC. Dysfunctional autophagy in RPE, a contributing factor in age-related macular degeneration. Cell Death Dis. 2018;8(1):e2537–e.

74. Seddon JM, Francis PJ, George S, Schultz DW, Rosner B, Klein ML. Association of CFH Y402H and LOC387715 A69S with progression of age-related macular degeneration. JAMA. 2007;297(16):1793–800. https://doi.org/10.1001/jama.297.16.1793.
75. Schaumberg DA, Hankinson SE, Guo Q, Rimm E, Hunter DJ. A prospective study of 2 major age-related macular degeneration susceptibility alleles and interactions with modifiable risk factors. Arch Ophthalmol. 2007;125(1):55–62. https://doi.org/10.1001/archopht.125.1.55.
76. Scott WK, Schmidt S, Hauser MA, Gallins P, Schnetz-Boutaud N, Spencer KL, Gilbert JR, Agarwal A, Postel EA, Haines JL, Pericak-Vance MA. Independent effects of complement factor H Y402H polymorphism and cigarette smoking on risk of age-related macular degeneration. Ophthalmology. 2007;114(6):1151–6. https://doi.org/10.1016/j.ophtha.2006.08.054.
77. Ebrahimi KB, Cano M, Rhee J, Datta S, Wang L, Handa JT. Oxidative stress induces an interactive decline in Wnt and Nrf2 signaling in degenerating retinal pigment epithelium. Antioxid Redox Signal. 2018;29(4):389–407. https://doi.org/10.1089/ars.2017.7084.
78. Thurman JM, Renner B, Kunchithapautham K, Ferreira VP, Pangburn MK, Ablonczy Z, Tomlinson S, Holers VM, Rohrer B. Oxidative stress renders retinal pigment epithelial cells susceptible to complement-mediated injury. J Biol Chem. 2009;284(25):16939–47. https://doi.org/10.1074/jbc.M808166200.
79. Kunchithapautham K, Atkinson C, Rohrer B. Smoke exposure causes Endoplasmic reticulum stress and lipid accumulation in retinal pigment epithelium through oxidative stress and complement activation. J Biol Chem. 2014;289(21):14534–46.
80. Ebrahimi KB, Fijalkowski N, Cano M, Handa JT. Decreased membrane complement regulators in the retinal pigmented epithelium contributes to age-related macular degeneration. J Pathol. 2013;229(5):729–42. https://doi.org/10.1002/path.4128.
81. Vogt SD, Curcio CA, Wang L, Li C-M, McGwin G Jr, Medeiros NE, Philp NJ, Kimble JA, Read RW. Retinal pigment epithelial expression of complement regulator CD46 is altered early in the course of geographic atrophy. Exp Eye Res. 2011;93(4):413–23.
82. Ferrington DA, Kapphahn RJ, Leary MM, Atilano SR, Terluk MR, Karunadharma P, Chen GK, Ratnapriya R, Swaroop A, Montezuma SR, Kenney MC. Increased retinal MtDNA damage in the CFH variant associated with age-related macular degeneration. Exp Eye Res. 2016;145:269–77. https://doi.org/10.1016/j.exer.2016.01.018.
83. La Cunza N, Tan LX, Thamban T, Germer CJ, Rathnasamy G, Toops KA, Lakkaraju A. Mitochondria-dependent phase separation of disease-relevant proteins drives pathological features of age-related macular degeneration. JCI Insight. 2021;6(9). https://doi.org/10.1172/jci.insight.142254.
84. Sparrow JR. Vitamin A-aldehyde adducts: AMD risk and targeted therapeutics. Proc Natl Acad Sci U S A. 2016;113(17):4564–9. https://doi.org/10.1073/pnas.1600474113.
85. Racz B, Varadi A, Kong J, Allikmets R, Pearson PG, Johnson G, Cioffi CL, Petrukhin K. A non-retinoid antagonist of retinol-binding protein 4 rescues phenotype in a model of Stargardt disease without inhibiting the visual cycle. J Biol Chem. 2018;293(29):11574–88.
86. Charbel Issa P, Barnard AR, Herrmann P, Washington I, MacLaren RE. Rescue of the Stargardt phenotype in Abca4 knockout mice through inhibition of vitamin A dimerization. Proceedings of the National Academy of Sciences. 2015;112(27):8415-20.
87. Ammar MJ, Hsu J, Chiang A, Ho AC, Regillo CD. Age-related macular degeneration therapy: a review. Curr Opin Ophthalmol. 2020;31(3):215–21. https://doi.org/10.1097/ICU.0000000000000657.
88. Ueda K, Zhao J, Kim HJ, Sparrow JR. Photodegradation of retinal bisretinoids in mouse models and implications for macular degeneration. Proc Natl Acad Sci U S A. 2016;113(25):6904–9. https://doi.org/10.1073/pnas.1524774113.
89. Lu W, Gomez NM, Lim JC, Guha S, O'Brien-Jenkins A, Coffey EE, Campagno KE, McCaughey SA, Laties AM, Carlsson LG, Mitchell CH. The P2Y(12) receptor antagonist Ticagrelor reduces lysosomal pH and autofluorescence in retinal pigmented epithelial cells from the ABCA4(-/-) mouse model of retinal degeneration. Front Pharmacol. 2018;9:242. https://doi.org/10.3389/fphar.2018.00242.
90. Saini JS, Corneo B, Miller JD, Kiehl TR, Wang Q, Boles NC, Blenkinsop TA, Stern JH, Temple S. Nicotinamide ameliorates disease phenotypes in a human iPSC model of Age-Related macular degeneration. Cell Stem Cell. 2017;20(5):635–e477. https://doi.org/10.1016/j.stem.2016.12.015.
91. Zhang M, Jiang N, Chu Y, Postnikova O, Varghese R, Horvath A, Cheema AK, Golestaneh N. Dysregulated metabolic pathways in age-related macular degeneration. Sci Rep. 2020;10(1):2464. https://doi.org/10.1038/s41598-020-59244-4.
92. van Leeuwen EM, Emri E, Merle BMJ, Colijn JM, Kersten E, Cougnard-Gregoire A, Dammeier S, Meester-Smoor M, Pool FM, de Jong EK, Delcourt C, Rodrigez-Bocanegra E, Biarnes M, Luthert PJ, Ueffing M, Klaver CCW, Nogoceke E, den Hollander AI, Lengyel I. A new perspective on lipid research in age-related macular degeneration. Prog Retin Eye Res. 2018;67:56–86. https://doi.org/10.1016/j.preteyeres.2018.04.006.
93. Paun CC, Ersoy L, Schick T, Groenewoud JM, Lechanteur YT, Fauser S, Hoyng CB, de Jong EK, den Hollander AI. Genetic variants and systemic complement activation levels are associated with serum lipoprotein levels in Age-Related macular degeneration. Invest Ophthalmol Vis Sci. 2015;56(13):7766–73. https://doi.org/10.1167/iovs.15-17035.
94. Malek G, Li CM, Guidry C, Medeiros NE, Curcio CA. Apolipoprotein B in cholesterol-containing Drusen and basal deposits of human eyes with age-related maculopathy. Am J Pathol. 2003;162(2):413–25. https://doi.org/10.1016/S0002-9440(10)63836-9.
95. Rudolf M, Malek G, Messinger JD, Clark ME, Wang L, Curcio CA. Sub-retinal drusenoid deposits in human retina: organization and composition. Exp Eye Res. 2008;87(5):402–8. https://doi.org/10.1016/j.exer.2008.07.010.
96. Li CM, Chung BH, Presley JB, Malek G, Zhang X, Dashti N, Li L, Chen J, Bradley K, Kruth HS, Curcio CA. Lipoprotein-like particles and cholesteryl esters in human Bruch's membrane: initial characterization. Invest Ophthalmol Vis Sci. 2005;46(7):2576–86. https://doi.org/10.1167/iovs.05-0034.
97. Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G. Apolipoprotein E and alzheimer disease: pathobiology and targeting strategies. Nat Rev Neurol. 2019;15(9):501–18. https://doi.org/10.1038/s41582-019-0228-7.
98. Leduc V, Domenger D, De Beaumont L, Lalonde D, Belanger-Jasmin S, Poirier J. Function and comorbidities of Apolipoprotein e in Alzheimer's disease. Int J Alzheimers Dis. 2011;2011:974361DOI. https://doi.org/10.4061/2011/974361.
99. Toops KA, Tan LX, Lakkaraju A, Apolipoprotein. E isoforms and AMD: Springer; 2016.
100. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, Van Duijn CM. Effects of age, sex, and ethnicity on the association between Apolipoprotein E genotype and alzheimer disease: a meta-analysis. JAMA. 1997;278(16):1349–56.
101. Zareparsi S, Reddick AC, Branham KE, Moore KB, Jessup L, Thoms S, Smith-Wheelock M, Yashar BM, Swaroop A. Association of Apolipoprotein E alleles with susceptibility to age-related macular degeneration in a large cohort from a single center. Invest Ophthalmol Vis Sci. 2004;45(5):1306. https://doi.org/10.1167/iovs.03-1253.
102. McKay GJ, Patterson CC, Chakravarthy U, Dasari S, Klaver CC, Vingerling JR, Ho L, de Jong PT, Fletcher AE, Young IS, Seland JH, Rahu M, Soubrane G, Tomazzoli L, Topouzis F, Vioque J, Hingorani AD, Sofat R, Dean M, Sawitzke J, Seddon JM, Peter I, Webster AR, Moore AT, Yates JR, Cipriani V, Fritsche LG, Weber BH, Keilhauer CN, Lotery AJ, Ennis S, Klein ML, Francis PJ, Stambolian D, Orlin A, Gorin MB, Weeks DE, Kuo CL, Swaroop A, Othman M, Kanda A, Chen W, Abecasis GR, Wright AF, Hayward C, Baird PN, Guymer RH, Attia J, Thakkinstian A, Silvestri G. Evidence of association of APOE with age-related macular degeneration: a pooled analysis of 15 studies. Hum Mutat. 2011;32(12):1407–16. https://doi.org/10.1002/humu.21577.
103. Wheeler RJ, Hyman AA. Controlling compartmentalization by non-membrane-bound organelles. Philos Trans R Soc Lond B Biol Sci. 2018;373(1747):20170193. https://doi.org/10.1098/rstb.2017.0193.
104. Molliex A, Temirov J, Lee J, Coughlin M, Kanagaraj AP, Kim HJ, Mittag T, Taylor JP. Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. Cell. 2015;163(1):123–33. https://doi.org/10.1016/j.cell.2015.09.015.
105. Handa JT, Bowes Rickman C, Dick AD, Gorin MB, Miller JW, Toth CA, Ueffing M, Zarbin M, Farrer LA. A systems biology approach towards Understanding and treating non-neovascular age-related macular degeneration. Nat Commun. 2019;10(1):3347. https://doi.org/10.1038/s41467-019-11262-1.
106. Roizenblatt M, Naranjit N, Maia M, Gehlbach PL. The question of a role for Statins in Age-Related macular degeneration. Int J Mol Sci. 2018;19(11):3688. https://doi.org/10.3390/ijms19113688.
107. Gehlbach P, Li T, Hatef E. Statins for age-related macular degeneration. Cochrane Database Syst Rev. 2016;2016(8):CD006927DOI. https://doi.org/10.1002/14651858.CD006927.pub5.
108. Vavvas DG, Daniels AB, Kapsala ZG, Goldfarb JW, Ganotakis E, Loewenstein JI, Young LH, Gragoudas ES, Eliott D, Kim IK. Regression of some high-risk features of age-related macular degeneration (AMD) in patients receiving intensive Statin treatment. EBioMedicine. 2016;5:198–203.

109. Choudhary M, Ismail EN, Yao P-L, Tayyari F, Radu RA, Nusinowitz S, Boulton ME, Apte RS, Ruberti JW, Handa JT. LXRs regulate features of age-related macular degeneration and May be a potential therapeutic target. JCI Insight. 2020;5(1).
110. Fessler MB. The challenges and promise of targeting the liver X receptors for treatment of inflammatory disease. Pharmacol Ther. 2018;181:1–12. https://doi.org/10.1016/j.pharmthera.2017.07.010.
111. Hannun YA, Obeid LM. Sphingolipids and their metabolism in physiology and disease. Nat Rev Mol Cell Biol. 2018;19(3):175–91.
112. Fan J, Wu BX, Crosson CE. Suppression of acid Sphingomyelinase protects the retina from ischemic injury. Invest Ophthalmol Vis Sci. 2016;57(10):4476–84. https://doi.org/10.1167/iovs.16-19717.
113. Simon MV, Basu SK, Qaladize B, Grambergs R, Rotstein NP, Mandal N. Sphingolipids as critical players in retinal physiology and pathology. J Lipid Res. 2021;62.
114. Strettoi E, Gargini C, Novelli E, Sala G, Piano I, Gasco P, Ghidoni R. Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of retinitis pigmentosa. Proc Natl Acad Sci U S A. 2010;107(43):18706–11. https://doi.org/10.1073/pnas.1007644107.
115. Kornhuber J, Muehlbacher M, Trapp S, Pechmann S, Friedl A, Reichel M, Muhle C, Terfloth L, Groemer TW, Spitzer GM, Liedl KR, Gulbins E, Tripal P. Identification of novel functional inhibitors of acid Sphingomyelinase. PLoS ONE. 2011;6(8):e23852. https://doi.org/10.1371/journal.pone.0023852.
116. Kornhuber J, Müller CP, Becker KA, Reichel M, Gulbins E. The ceramide system as a novel antidepressant target. Trends Pharmacol Sci. 2014;35(6):293–304.
117. van Leeuwen R, Tomany SC, Wang JJ, Klein R, Mitchell P, Hofman A, Klein BE, Vingerling JR, Cumming RG, de Jong PT. Is medication use associated with the incidence of early age-related maculopathy? Pooled findings from 3 continents. Ophthalmology. 2004;111(6):1169–75.
118. Klein R, Klein BE, Jensen SC, Cruickshanks KJ, Lee KE, Danforth LG, Tomany SC. Medication use and the 5-year incidence of early age-related maculopathy: the beaver dam eye study. Arch Ophthalmol. 2001;119(9):1354–9. https://doi.org/10.1001/archopht.119.9.1354.
119. Park MH, Lee JY, Park KH, Jung IK, Kim K-T, Lee Y-S, Ryu H-H, Jeong Y, Kang M, Schwaninger M. Vascular and neurogenic rejuvenation in aging mice by modulation of ASM. Neuron. 2018;100(1):167–82. e9.
120. Lin G, Wang L, Marcogliese PC, Bellen HJ. Sphingolipids in the pathogenesis of Parkinson's disease and parkinsonism. Trends Endocrinol Metab. 2019;30(2):106–17. https://doi.org/10.1016/j.tem.2018.11.003.
121. de Guimaraes TAC, Georgiou M, Bainbridge JW, Michaelides M. Gene therapy for neovascular age-related macular degeneration: rationale, clinical trials and future directions. Br J Ophthalmol. 2021;105(2):151–7.
122. Morral N, O'Neal W, Zhou H, Langston C, Beaudet A. Immune responses to reporter proteins and high viral dose limit duration of expression with adenoviral vectors: comparison of E2a wild type and E2a deleted vectors. Hum Gene Ther. 1997;8(10):1275–86. https://doi.org/10.1089/hum.1997.8.10-1275.
123. Gao GP, Yang Y, Wilson JM. Biology of adenovirus vectors with E1 and E4 deletions for liver-directed gene therapy. J Virol. 1996;70(12):8934–43. https://doi.org/10.1128/JVI.70.12.8934-8943.1996.
124. Morral N, O'Neal W, Rice K, Leland M, Kaplan J, Piedra PA, Zhou H, Parks RJ, Velji R, Aguilar-Cordova E, Wadsworth S, Graham FL, Kochanek S, Carey KD, Beaudet AL. Administration of helper-dependent adenoviral vectors and sequential delivery of different vector serotype for long-term liver-directed gene transfer in baboons. Proc Natl Acad Sci U S A. 1999;96(22):12816–21. https://doi.org/10.1073/pnas.96.22.12816.
125. Morral N, Parks RJ, Zhou H, Langston C, Schiedner G, Quinones J, Graham FL, Kochanek S, Beaudet AL. High doses of a helper-dependent adenoviral vector yield Supraphysiological levels of α 1-antitrypsin with negligible toxicity. Hum Gene Ther. 1998;9(18):2709–16.
126. Lisowski L, Tay SS, Alexander IE. Adeno-associated virus serotypes for gene therapeutics. Curr Opin Pharmacol. 2015;24:59–67. https://doi.org/10.1016/j.coph.2015.07.006.
127. Everson EM, Trobridge GD. Retroviral vector interactions with hematopoietic cells. Curr Opin Virol. 2016;21:41–6. https://doi.org/10.1016/j.coviro.2016.07.010.
128. Kumar M, Keller B, Makalou N, Sutton RE. Systematic determination of the packaging limit of lentiviral vectors. Hum Gene Ther. 2001;12(15):1893–905. https://doi.org/10.1089/104303401753153947.
129. Booth C, Gaspar HB, Thrasher AJ. Treating immunodeficiency through HSC gene therapy. Trends Mol Med. 2016;22(4):317–27.
130. Zufferey R, Dull T, Mandel RJ, Bukovsky A, Quiroz D, Naldini L, Trono D. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. J Virol. 1998;72(12):9873–80. https://doi.org/10.1128/JVI.72.12.9873-9880.1998.
131. Shaw A, Cornetta K. Design and potential of Non-Integrating lentiviral vectors. Biomedicines. 2014;2(1):14–35. https://doi.org/10.3390/biomedicines2010014.
132. Stieger K, Cronin T, Bennett J, Rolling F. Adeno-associated virus mediated gene therapy for retinal degenerative diseases. Methods Mol Biol. 2011;807:179–218. https://doi.org/10.1007/978-1-61779-370-7_8.
133. Surace EM, Auricchio A. Versatility of AAV vectors for retinal gene transfer. Vis Res. 2008;48(3):353–9. https://doi.org/10.1016/j.visres.2007.07.027.
134. Hareendran S, Balakrishnan B, Sen D, Kumar S, Srivastava A, Jayandharan GR. Adeno-associated virus (AAV) vectors in gene therapy: immune challenges and strategies to circumvent them. Rev Med Virol. 2013;23(6):399–413.
135. Wang JH, Zhan W, Gallagher TL, Gao G. Recombinant adeno-associated virus as a delivery platform for ocular gene therapy: A comprehensive review. Mol Ther. 2024;32(12):4185–207. https://doi.org/10.1016/j.ymthe.2024.10.017.
136. Kolesnik VV, Nurtdinov RF, Oloruntimehin ES, Karabelsky AV, Malogolovkin AS. Optimization strategies and advances in the research and development of AAV-based gene therapy to deliver large transgenes. Clin Transl Med. 2024;14(3):e1607. https://doi.org/10.1002/ctm2.1607.
137. Yang B, Li G, Liu J, Li X, Zhang S, Sun F, Liu W. Nanotechnology for Age-Related macular degeneration. Pharmaceutics. 2021;13(12):2035. https://doi.org/10.3390/pharmaceutics13122035.
138. Finn JD, Smith AR, Patel MC, Shaw L, Youniss MR, van Heteren J, Dirstine T, Ciullo C, Lescarbeau R, Seitzer J, Shah RR, Shah A, Ling D, Growe J, Pink M, Rohde E, Wood KM, Salomon WE, Harrington WF, Dombrowski C, Strapps WR, Chang Y, Morrissey DV. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. Cell Rep. 2018;22(9):2227–35. https://doi.org/10.1016/j.celrep.2018.02.014.
139. Sun D, Sun Z, Jiang H, Vaidya AM, Xin R, Ayat NR, Schilb AL, Qiao PL, Han Z, Naderi A. Synthesis and evaluation of pH-sensitive multifunctional lipids for efficient delivery of CRISPR/Cas9 in gene editing. Bioconjug Chem. 2018;30(3):667–78.
140. del Pozo-Rodríguez A, Delgado D, Gascón AR, Solinís MÁ. Lipid nanoparticles as drug/gene delivery systems to the retina. J Ocul Pharmacol Ther. 2013;29(2):173–88.
141. Wang Y, Rajala A, Rajala RV. Lipid nanoparticles for ocular gene delivery. J Funct Biomater. 2015;6(2):379–94. https://doi.org/10.3390/jfb6020379.
142. Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv. 2018;25(1):1234–57. https://doi.org/10.1080/10717544.2018.1474964.
143. Kang G-Y, Bang JY, Choi AJ, Yoon J, Lee W-C, Choi S, Yoon S, Kim HC, Baek J-H, Park HS. Exosomal proteins in the aqueous humor as novel biomarkers in patients with neovascular age-related macular degeneration. J Proteome Res. 2014;13(2):581–95.
144. Biasutto L, Chiechi A, Couch R, Liotta LA, Espina V. Retinal pigment epithelium (RPE) exosomes contain signaling phosphoproteins affected by oxidative stress. Exp Cell Res. 2013;319(13):2113–23. https://doi.org/10.1016/j.yexcr.2013.05.005.
145. Hajrasouliha AR, Jiang G, Lu Q, Lu H, Kaplan HJ, Zhang H-G, Shao H. Exosomes from retinal astrocytes contain antiangiogenic components that inhibit laser-induced choroidal neovascularization. J Biol Chem. 2013;288(39):28058–67.
146. Duan L, Ouyang K, Wang J, Xu L, Xu X, Wen C, Xie Y, Liang Y, Xia J. Exosomes as targeted delivery platform of CRISPR/Cas9 for therapeutic genome editing. ChemBioChem. 2021;22(24):3360–8.
147. Lamichhane TN, Raiker RS, Jay SM. Exogenous DNA loading into extracellular vesicles via electroporation is size-dependent and enables limited gene delivery. Mol Pharm. 2015;12(10):3650–7.
148. Nazari H, Zhang L, Zhu D, Chader GJ, Falabella P, Stefanini F, Rowland T, Clegg DO, Kashani AH, Hinton DR, Humayun MS. Stem cell based therapies for age-related macular degeneration: the promises and the challenges. Prog Retin Eye Res. 2015;48:1–39. https://doi.org/10.1016/j.preteyeres.2015.06.004.
149. Schwartz SD, Regillo CD, Lam BL, Eliott D, Rosenfeld PJ, Gregori NZ, Hubschman JP, Davis JL, Heilwell G, Spirn M, Maguire J, Gay R, Bateman J, Ostrick RM, Morris D, Vincent M, Anglade E, Del Priore LV, Lanza R. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. Lancet. 2015;385(9967):509–16. https://doi.org/10.1016/S0140-6736(14)61376-3.
150. Lund RD, Wang S, Klimanskaya I, Holmes T, Ramos-Kelsey R, Lu B, Girman S, Bischoff N, Sauve Y, Lanza R. Human embryonic stem cell-derived cells rescue

visual function in dystrophic RCS rats. Cloning Stem Cells. 2006;8(3):189–99. https://doi.org/10.1089/clo.2006.8.189.
151. Lund RD, Wang S, Lu B, Girman S, Holmes T, Sauve Y, Messina DJ, Harris IR, Kihm AJ, Harmon AM, Chin FY, Gosiewska A, Mistry SK. Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease. Stem Cells. 2007;25(3):602–11. https://doi.org/10.1634/stemcells.2006-0308.
152. Lu B, Malcuit C, Wang S, Girman S, Francis P, Lemieux L, Lanza R, Lund R. Long-term safety and function of RPE from human embryonic stem cells in preclinical models of macular degeneration. Stem Cells. 2009;27(9):2126–35. https://doi.org/10.1002/stem.149.
153. Kamao H, Mandai M, Okamoto S, Sakai N, Suga A, Sugita S, Kiryu J, Takahashi M. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. Stem Cell Rep. 2014;2(2):205–18. https://doi.org/10.1016/j.stemcr.2013.12.007.
154. Lu B, Tai YC, Humayun MS. Microdevice-based cell therapy for age-related macular degeneration. Dev Ophthalmol. 2014;53:155–66. https://doi.org/10.1159/000357375.
155. Mooney SM, Grande JP, Salisbury JL, Janknecht R. Sumoylation of p68 and p72 RNA helicases affects protein stability and transactivation potential. Biochemistry. 2010;49(1):1–10. https://doi.org/10.1021/bi901263m.
156. Fox IJ, Daley GQ, Goldman SA, Huard J, Kamp TJ, Trucco M. Stem cell therapy. Use of differentiated pluripotent stem cells as replacement therapy for treating disease. Science. 2014;345(6199):1247391. https://doi.org/10.1126/science.1247391.
157. Ke X, Wu Y, Yu X, Yang S, Luo S, Qing X, Ingerman A, Oughton A, Shearn SP, Zheng Q. Combined complement inhibitors and Anti-VEGF therapy for the treatment of dry Age-Related macular degeneration (AMD). Investig Ophthalmol Vis Sci. 2024;65(7):5317.
158. Calton MA, Croze RH, Burns C, Beliakoff G, Vazin T, Szymanski P, Schmitt C, Klein A, Leong M, Quezada M, Holt J, Bolender G, Barglow K, Khoday D, Mason T, Delaria K, Hassanipour M, Kotterman M, Khanani AM, Schaffer D, Francis P, Kirn D. Design and characterization of a novel intravitreal Dual-Transgene genetic medicine for neovascular retinopathies. Invest Ophthalmol Vis Sci. 2024;65(14):1. https://doi.org/10.1167/iovs.65.14.1.
159. Kotterman M, Beliakoff G, Croze R, Vazin T, Schmitt C, Szymanski P, Leong M, Quezada M, Holt J, Barglow K. Directed evolution of AAV targeting primate retina by intravitreal injection identifies R100, a variant demonstrating robust gene delivery and therapeutic efficacy in non-human primates. BioRxiv. 2021:2021.06. 24.449775.
160. Johari YB, Mercer AC, Liu Y, Brown AJ, James DC. Design of synthetic promoters for controlled expression of therapeutic genes in retinal pigment epithelial cells. Biotechnol Bioeng. 2021;118(5):2001–15. https://doi.org/10.1002/bit.27713.
161. Nashine S, Kenney MC. Further Understanding of epigenetic dysfunction of the retinal pigment epithelium in AMD. Expert Rev Ophthalmol. 2020;15(4):221–31.
162. Chan YK, Dick AD, Hall SM, Langmann T, Scribner CL, Mansfield BC. Ocular gene therapy inflammation working G. inflammation in viral Vector-Mediated ocular gene therapy: A review and report from a workshop hosted by the foundation fighting blindness, 9/2020. Transl Vis Sci Technol. 2021;10(4):3. https://doi.org/10.1167/tvst.10.4.3.
163. Blasiak J, Pawlowska E, Ciupińska J, Derwich M, Szczepanska J, Kaarniranta K. A new generation of gene therapies as the future of wet AMD treatment. Int J Mol Sci. 2024;25(4):2386.
164. Paremskaia AI, Kogan AA, Murashkina A, Naumova DA, Satish A, Abramov IS, Feoktistova SG, Mityaeva ON, Deviatkin AA, Volchkov PY. Codon-optimization in gene therapy: promises, prospects and challenges. Front Bioeng Biotechnol. 2024;12:1371596. https://doi.org/10.3389/fbioe.2024.1371596.
165. Wang J-H, Gessler DJ, Zhan W, Gallagher TL, Gao G. Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduct Target Therapy. 2024;9(1):78.
166. Trincao-Marques J, Ayton LN, Hickey DG, Marques-Neves C, Guymer RH, Edwards TL, Sousa DC. Gene and cell therapy for age-related macular degeneration: A review. Surv Ophthalmol. 2024;69(5):665–76. https://doi.org/10.1016/j.survophthal.2024.05.002.
167. Mori K, Duh E, Gehlbach P, Ando A, Takahashi K, Pearlman J, Mori K, Yang HS, Zack DJ, Ettyreddy D, Brough DE, Wei LL, Campochiaro PA. Pigment epithelium-derived factor inhibits retinal and choroidal neovascularization. J Cell Physiol. 2001;188(2):253–63. https://doi.org/10.1002/jcp.1114.
168. Mori K, Gehlbach P, Ando A, McVey D, Wei L, Campochiaro PA. Regression of ocular neovascularization in response to increased expression of pigment epithelium-derived factor. Invest Ophthalmol Vis Sci. 2002;43(7):2428–34.
169. Campochiaro PA, Nguyen QD, Shah SM, Klein ML, Holz E, Frank RN, Saperstein DA, Gupta A, Stout JT, Macko J, DiBartolomeo R, Wei LL. Adenoviral vector-delivered pigment epithelium-derived factor for neovascular age-related macular degeneration: results of a phase I clinical trial. Hum Gene Ther. 2006;17(2):167–76. https://doi.org/10.1089/hum.2006.17.167.
170. Campochiaro PA, Lauer AK, Sohn EH, Mir TA, Naylor S, Anderton MC, Kelleher M, Harrop R, Ellis S, Mitrophanous KA. Lentiviral vector gene transfer of endostatin/angiostatin for macular degeneration (GEM) study. Hum Gene Ther. 2017;28(1):99–111. https://doi.org/10.1089/hum.2016.117.
171. Chen J, Lin FL, Leung JYK, Tu L, Wang JH, Chuang YF, Li F, Shen HH, Dusting GJ, Wong VHY, Lisowski L, Hewitt AW, Bui BV, Zhong J, Liu GS. A drug-tunable Flt23k gene therapy for controlled intervention in retinal neovascularization. Angiogenesis. 2021;24(1):97–110. https://doi.org/10.1007/s10456-020-09745-7.
172. Esteve-Rudd J, Francis J, Hughes J, Ellis S. Preclinical Pharmacology and safety of GT005, an investigational gene therapy targeting the complement pathway for the treatment of geographic atrophy. Investig Ophthalmol Vis Sci. 2022;63(7):294–F. 0097-294–F.
173. Heier JS, Cohen MN, Chao DL, Pepio A, Shiraga Y, Capuano G, Rogers A, Ackert J, Sen HN, Csaky K. Phase 1 study of JNJ-81201887 gene therapy in geographic atrophy secondary to Age-Related macular degeneration. Ophthalmology. 2024;131(12):1377–88. https://doi.org/10.1016/j.ophtha.2024.06.013.
174. Castro BFM, Steel JC, Layton CJ. AAV-Based strategies for treatment of retinal and choroidal vascular diseases: advances in Age-Related macular degeneration and diabetic retinopathy therapies. BioDrugs. 2024;38(1):73–93. https://doi.org/10.1007/s40259-023-00629-y.
175. Silva R, Arias L, Nunes S, Farinha C, Coimbra R, Marques JP, Cachulo ML, Figueira J, Barreto P, Madeira MH, Pires I, Sousa JC, Distefano L, Rosa P, Carneiro A, Vaz-Pereira S, Meireles A, Cabrera F, Bures A, Mendonca L, Fernandez-Vega-Sanz A, Barrao S, Koh A, Cheung CMG, Cunha-Vaz JG, Murta J, Group EVnAS. Efficacy and safety of intravitreal Aflibercept treat and extend for polypoidal choroidal vasculopathy in the ATLANTIC study: A randomized clinical trial. Ophthalmologica. 2022;245(1):80–90. https://doi.org/10.1159/000518235.
176. Mehta N, Robbins DA, Yiu G. Ocular inflammation and treatment emergent adverse events in retinal gene therapy. Int Ophthalmol Clin. 2021;61(3):151–77. https://doi.org/10.1097/IIO.0000000000000366.
177. Zaiss AK, Liu Q, Bowen GP, Wong NC, Bartlett JS, Muruve DA. Differential activation of innate immune responses by adenovirus and adeno-associated virus vectors. J Virol. 2002;76(9):4580–90. https://doi.org/10.1128/jvi.76.9.4580-4590.2002.
178. Heier JS, Kherani S, Desai S, Dugel P, Kaushal S, Cheng SH, Delacono C, Purvis A, Richards S, Le-Halpere A, Connelly J, Wadsworth SC, Varona R, Buggage R, Scaria A, Campochiaro PA. Intravitreous injection of AAV2-sFLT01 in patients with advanced neovascular age-related macular degeneration: a phase 1, open-label trial. Lancet. 2017;390(10089):50–61. https://doi.org/10.1016/S0140-6736(17)30979-0.
179. Reichel FF, Seitz I, Wozar F, Dimopoulos S, Jung R, Kempf M, Kohl S, Kortum FC, Ott S, Pohl L, Stingl K, Bartz-Schmidt KU, Stingl K, Fischer MD. Development of retinal atrophy after subretinal gene therapy with voretigene neparvovec. Br J Ophthalmol. 2023;107(9):1331–5. https://doi.org/10.1136/bjophthalmol-2021-321023.
180. Walters L, Palmer JG. The ethics of human gene therapy. Science. 1986;320.
181. Shirley JL, de Jong YP, Terhorst C, Herzog RW. Immune responses to viral gene therapy vectors. Mol Ther. 2020;28(3):709–22. https://doi.org/10.1016/j.ymthe.2020.01.001.
182. Guedan S, Calderon H, Posey AD Jr., Maus MV. Engineering and design of chimeric antigen receptors. Mol Ther Methods Clin Dev. 2019;12:145–56. https://doi.org/10.1016/j.omtm.2018.12.009.
183. Wang X, Herzog RW, Byrne BJ, Kumar SRP, Zhou Q, Buchholz CJ, Biswas M. Immune modulatory cell therapy for hemophilia B based on CD20-Targeted lentiviral gene transfer to primary B cells. Mol Ther Methods Clin Dev. 2017;5:76–82. https://doi.org/10.1016/j.omtm.2017.03.005.
184. Frank AM, Buchholz CJ. Surface-engineered lentiviral vectors for selective gene transfer into subtypes of lymphocytes. Mol Therapy-Methods Clin Dev. 2019;12:19–31.
185. Jamali A, Kapitza L, Schaser T, Johnston ICD, Buchholz CJ, Hartmann J. Highly efficient and selective CAR-Gene transfer using CD4- and CD8-Targeted

lentiviral vectors. Mol Ther Methods Clin Dev. 2019;13:371–9. https://doi.org/10.1016/j.omtm.2019.03.003.
186. Milani M, Annoni A, Moalli F, Liu T, Cesana D, Calabria A, Bartolaccini S, Biffi M, Russo F, Visigalli I, Raimondi A, Patarroyo-White S, Drager D, Cristofori P, Ayuso E, Montini E, Peters R, Iannacone M, Cantore A, Naldini L. Phagocytosis-shielded lentiviral vectors improve liver gene therapy in nonhuman primates. Sci Transl Med. 2019;11(493):eaav7325. https://doi.org/10.1126/scitranslmed.aav7325.
187. Brown BD, Sitia G, Annoni A, Hauben E, Sergi LS, Zingale A, Roncarolo MG, Guidotti LG, Naldini L. In vivo administration of lentiviral vectors triggers a type I interferon response that restricts hepatocyte gene transfer and promotes vector clearance. Blood. 2007;109(7):2797–805.
188. Agudo J, Ruzo A, Kitur K, Sachidanandam R, Blander JM, Brown BD. A TLR and non-TLR mediated innate response to lentiviruses restricts hepatocyte entry and can be ameliorated by Pharmacological Blockade. Mol Ther. 2012;20(12):2257–67.
189. Borsotti C, Borroni E, Follenzi A. Lentiviral vector interactions with the host cell. Curr Opin Virol. 2016;21:102–8. https://doi.org/10.1016/j.coviro.2016.08.016.
190. Rossetti M, Gregori S, Hauben E, Brown BD, Sergi LS, Naldini L, Roncarolo M-G. HIV-1-derived lentiviral vectors directly activate plasmacytoid dendritic cells, which in turn induce the maturation of myeloid dendritic cells. Hum Gene Ther. 2011;22(2):177–88.
191. Pichlmair A, Diebold SS, Gschmeissner S, Takeuchi Y, Ikeda Y, Collins MK, Reis e Sousa C. Tubulovesicular structures within vesicular stomatitis virus G protein-pseudotyped lentiviral vector preparations carry DNA and stimulate antiviral responses via Toll-like receptor 9. J Virol. 2007;81(2):539–47.
192. Agudo J, Ruzo A, Tung N, Salmon H, Leboeuf M, Hashimoto D, Becker C, Garrett-Sinha LA, Baccarini A, Merad M, Brown BD. The miR-126-VEGFR2 axis controls the innate response to pathogen-associated nucleic acids. Nat Immunol. 2014;15(1):54–62. https://doi.org/10.1038/ni.2767.
193. Rogers GL, Herzog RW. One MicroRNA controls both angiogenesis and TLR-mediated innate immunity to nucleic acids. Mol Ther. 2014;22(2):249–50.
194. Gao D, Wu J, Wu Y-T, Du F, Aroh C, Yan N, Sun L, Chen ZJ. Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. Science. 2013;341(6148):903–6.
195. Lahaye X, Satoh T, Gentili M, Cerboni S, Conrad C, Hurbain I, El Marjou A, Lacabaratz C, Lelievre JD, Manel N. The capsids of HIV-1 and HIV-2 determine immune detection of the viral cDNA by the innate sensor cGAS in dendritic cells. Immunity. 2013;39(6):1132–42. https://doi.org/10.1016/j.immuni.2013.11.002.
196. Khanani AM, Thomas MJ, Aziz AA, Weng CY, Danzig CJ, Yiu G, Kiss S, Waheed NK, Kaiser PK. Review of gene therapies for age-related macular degeneration. Eye (Lond). 2022;36(2):303–11. https://doi.org/10.1038/s41433-021-01842-1.
197. Campochiaro PA, Avery R, Brown DM, Heier JS, Ho AC, Huddleston SM, Jaffe GJ, Khanani AM, Pakola S, Pieramici DJ, Wykoff CC, Van Everen S. Gene therapy for neovascular age-related macular degeneration by subretinal delivery of RGX-314: a phase 1/2a dose-escalation study. Lancet. 2024;403(10436):1563–73. https://doi.org/10.1016/S0140-6736(24)00310-6.
198. Nussenblatt RB, Lee RW, Chew E, Wei L, Liu B, Sen HN, Dick AD, Ferris FL. Immune responses in age-related macular degeneration and a possible long-term therapeutic strategy for prevention. Am J Ophthalmol. 2014;158(1):5–e112. https://doi.org/10.1016/j.ajo.2014.03.014.
199. Cheng SY, Punzo C. Update on viral gene therapy clinical trials for retinal diseases. Hum Gene Ther. 2022;33(17–18):865–78. https://doi.org/10.1089/hum.2022.159.
200. Vandendriessche T, Thorrez L, Acosta-Sanchez A, Petrus I, Wang L, Ma L, L DEW, Iwasaki Y, Gillijns V, Wilson JM, Collen D, Chuah MK. Efficacy and safety of adeno-associated viral vectors based on serotype 8 and 9 vs. lentiviral vectors for hemophilia B gene therapy. J Thromb Haemost. 2007;5(1):16–24. https://doi.org/10.1111/j.1538-7836.2006.02220.x.
201. Yang TY, Braun M, Lembke W, McBlane F, Kamerud J, DeWall S, Tarcsa E, Fang X, Hofer L, Kavita U, Upreti VV, Gupta S, Loo L, Johnson AJ, Chandode RK, Stubenrauch KG, Vinzing M, Xia CQ, Jawa V. Immunogenicity assessment of AAV-based gene therapies: an IQ consortium industry white paper. Mol Ther Methods Clin Dev. 2022;26:471–94. https://doi.org/10.1016/j.omtm.2022.07.018.
202. Chandler S, Campochiaro P, Lauer A, Sohn EH, Stout T, Kelleher M, Harrop R, Ellis S, Mitrophanous K. Results from the phase i GEM study, evaluating safety and tolerability of subretinally-injected lentiviral vector (retinostat®) for the treatment of wet age-related macular degeneration (AMD). Investig Ophthalmol Vis Sci. 2015;56(7):2284.
203. Annoni A, Gregori S, Naldini L, Cantore A. Modulation of immune responses in lentiviral vector-mediated gene transfer. Cell Immunol. 2019;342:103802DOI. https://doi.org/10.1016/j.cellimm.2018.04.012.
204. Abordo-Adesida E, Follenzi A, Barcia C, Sciascia S, Castro MG, Naldini L, Lowenstein PR. Stability of lentiviral vector-mediated transgene expression in the brain in the presence of systemic antivector immune responses. Hum Gene Ther. 2005;16(6):741–51. https://doi.org/10.1089/hum.2005.16.741.
205. Leroy BP, Fischer MD, Flannery JG, MacLaren RE, Dalkara D, Scholl HPN, Chung DC, Spera C, Viriato D, Banhazi J. Gene therapy for inherited retinal disease: Long-Term durability of effect. Ophthalmic Res. 2023;66(1):179–96. https://doi.org/10.1159/000526317.
206. Zhang T, Enemchukwu NO, Jones A, Wang S, Dennis E, Watt CB, Pugh EN Jr., Fu Y. Genetic deletion of S-opsin prevents rapid cone degeneration in a mouse model of leber congenital amaurosis. Hum Mol Genet. 2015;24(6):1755–63. https://doi.org/10.1093/hmg/ddu588.
207. Jacobson SG, Cideciyan AV, Ratnakaram R, Heon E, Schwartz SB, Roman AJ, Peden MC, Aleman TS, Boye SL, Sumaroka A, Conlon TJ, Calcedo R, Pang JJ, Erger KE, Olivares MB, Mullins CL, Swider M, Kaushal S, Feuer WJ, Iannaccone A, Fishman GA, Stone EM, Byrne BJ, Hauswirth WW. Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. Arch Ophthalmol. 2012;130(1):9–24. https://doi.org/10.1001/archophthalmol.2011.298.
208. Chung DC, Lee K, Reape KZ, High KA, Lacey S, Viriato D. Long-Term effect of voretigene neparvovec on the Full-Field light sensitivity threshold test of patients with RPE65 mutation-associated inherited retinal dystrophy–post hoc analysis of phase I trial data. Investig Ophthalmol Vis Sci. 2019;60(9):3398.
209. Jacobson SG, Boye SL, Aleman TS, Conlon TJ, Zeiss CJ, Roman AJ, Cideciyan AV, Schwartz SB, Komaromy AM, Doobrajh M, Cheung AY, Sumaroka A, Pearce-Kelling SE, Aguirre GD, Kaushal S, Maguire AM, Flotte TR, Hauswirth WW. Safety in nonhuman primates of ocular AAV2-RPE65, a candidate treatment for blindness in leber congenital amaurosis. Hum Gene Ther. 2006;17(8):845–58. https://doi.org/10.1089/hum.2006.17.845.
210. Carvalho LS, Xu J, Pearson RA, Smith AJ, Bainbridge JW, Morris LM, Fliesler SJ, Ding XQ, Ali RR. Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. Hum Mol Genet. 2011;20(16):3161–75. https://doi.org/10.1093/hmg/ddr218.
211. Min S-H, Ruan Q, Boye SL, Boye S, Hauswirth WW, Kay CN. Expression of CNGB3 provides long-term rescue in the cone-only CNGB3 X NRL double knock-out (DKO) mouse. Investig Ophthalmol Vis Sci. 2014;55(13):3309.
212. Ye GJ, Budzynski E, Sonnentag P, Nork TM, Miller PE, McPherson L, Ver Hoeve JN, Smith LM, Arndt T, Mandapati S, Robinson PM, Calcedo R, Knop DR, Hauswirth WW, Chulay JD. Safety and biodistribution evaluation in CNGB3-Deficient mice of rAAV2tYF-PR1.7-hCNGB3, a Recombinant AAV vector for treatment of achromatopsia. Hum Gene Ther Clin Dev. 2016;27(1):27–36. https://doi.org/10.1089/humc.2015.163.
213. Ye GJ, Budzynski E, Sonnentag P, Nork TM, Sheibani N, Gurel Z, Boye SL, Peterson JJ, Boye SE, Hauswirth WW, Chulay JD. Cone-Specific promoters for gene therapy of achromatopsia and other retinal diseases. Hum Gene Ther. 2016;27(1):72–82. https://doi.org/10.1089/hum.2015.130.
214. Zhang Y, Wang S, Xu M, Pang J, Yuan Z, Zhao C. AAV-mediated human CNGB3 restores cone function in an all-cone mouse model of CNGB3 achromatopsia. J Biomedical Res. 2020;34(2):114.
215. Komaromy AM, Alexander JJ, Rowlan JS, Garcia MM, Chiodo VA, Kaya A, Tanaka JC, Acland GM, Hauswirth WW, Aguirre GD. Gene therapy rescues cone function in congenital achromatopsia. Hum Mol Genet. 2010;19(13):2581–93. https://doi.org/10.1093/hmg/ddq136.
216. Komaromy AM, Rowlan JS, Corr AT, Reinstein SL, Boye SL, Cooper AE, Gonzalez A, Levy B, Wen R, Hauswirth WW, Beltran WA, Aguirre GD. Transient photoreceptor Deconstruction by CNTF enhances rAAV-mediated cone functional rescue in late stage CNGB3-achromatopsia. Mol Ther. 2013;21(6):1131–41. https://doi.org/10.1038/mt.2013.50.
217. Ye GJ, Komaromy AM, Zeiss C, Calcedo R, Harman CD, Koehl KL, Stewart GA, Iwabe S, Chiodo VA, Hauswirth WW, Aguirre GD, Chulay JD. Safety and efficacy of AAV5 vectors expressing human or canine CNGB3 in CNGB3-Mutant dogs. Hum Gene Ther Clin Dev. 2017;28(4):197–207. https://doi.org/10.1089/humc.2017.125.
218. Ye GJ, Budzynski E, Sonnentag P, Nork TM, Miller PE, Sharma AK, Ver Hoeve JN, Smith LM, Arndt T, Calcedo R, Gaskin C, Robinson PM, Knop DR, Hauswirth WW, Chulay JD. Safety and biodistribution evaluation in Cynomolgus

macaques of rAAV2tYF-PR1.7-hCNGB3, a Recombinant AAV vector for treatment of achromatopsia. Hum Gene Ther Clin Dev. 2016;27(1):37–48. https://doi.org/10.1089/humc.2015.164.
219. Koch S, Sothilingam V, Garcia Garrido M, Tanimoto N, Becirovic E, Koch F, Seide C, Beck SC, Seeliger MW, Biel M, Muhlfriedel R, Michalakis S. Gene therapy restores vision and delays degeneration in the CNGB1(-/-) mouse model of retinitis pigmentosa. Hum Mol Genet. 2012;21(20):4486–96. https://doi.org/10.1093/hmg/dds290.
220. Michalakis S, Koch S, Sothilingam V, Garrido MG, Tanimoto N, Schulze E, Becirovic E, Koch F, Seide C, Beck SC, editors. Gene therapy restores vision and delays degeneration in the CNGB1–/– mouse model of retinitis pigmentosa. Retinal Degenerative Diseases: Mechanisms and Experimental Therapy; 2014.
221. Schon C, Sothilingam V, Muhlfriedel R, Garcia Garrido M, Beck SC, Tanimoto N, Wissinger B, Paquet-Durand F, Biel M, Michalakis S, Seeliger MW. Gene therapy successfully delays degeneration in a mouse model of PDE6A-Linked retinitis pigmentosa (RP43). Hum Gene Ther. 2017;28(12):1180–8. https://doi.org/10.1089/hum.2017.156.
222. Wert KJ, Sancho-Pelluz J, Tsang SH. Mid-stage intervention achieves similar efficacy as conventional early-stage treatment using gene therapy in a preclinical model of retinitis pigmentosa. Hum Mol Genet. 2014;23(2):514–23.
223. Mowat FM, Occelli LM, Bartoe JT, Gervais KJ, Bruewer AR, Querubin J, Dinculescu A, Boye SL, Hauswirth WW, Petersen-Jones SM. Gene therapy in a large animal model of PDE6A-Retinitis pigmentosa. Front Neurosci. 2017;11:342. https://doi.org/10.3389/fnins.2017.00342.
224. Occelli LM, Schon C, Seeliger MW, Biel M, Michalakis S, Petersen-Jones SM. Gene supplementation rescues rod function and preserves photoreceptor and retinal morphology in dogs, leading the way toward treating human PDE6A-Retinitis pigmentosa. Hum Gene Ther. 2017;28(12):1189–201. https://doi.org/10.1089/hum.2017.155.
225. Peters T, Wilhelm B, Korte S, Bartz-Schmidt KU, Michalakis S, Ochakovski GA, Fischer DM. Evaluation of electroretinography in cynomolgus monkeys after subretinal injection of rAAV. hPDE6A. Investig Ophthalmol Vis Sci. 2017;58(8):4096.
226. Nishiguchi KM, Carvalho LS, Rizzi M, Powell K, Holthaus SM, Azam SA, Duran Y, Ribeiro J, Luhmann UF, Bainbridge JW, Smith AJ, Ali RR. Gene therapy restores vision in rd1 mice after removal of a confounding mutation in Gpr179. Nat Commun. 2015;6(1):6006. https://doi.org/10.1038/ncomms7006.
227. Pang JJ, Boye SL, Kumar A, Dinculescu A, Deng W, Li J, Li Q, Rani A, Foster TC, Chang B, Hawes NL, Boatright JH, Hauswirth WW. AAV-mediated gene therapy for retinal degeneration in the rd10 mouse containing a recessive PDEbeta mutation. Invest Ophthalmol Vis Sci. 2008;49(10):4278–83. https://doi.org/10.1167/iovs.07-1622.
228. Ameri H. Prospect of retinal gene therapy following commercialization of voretigene neparvovec-rzyl for retinal dystrophy mediated by RPE65 mutation. J Curr Ophthalmol. 2018;30(1):1–2. https://doi.org/10.1016/j.joco.2018.01.006.
229. Kwon I, Schaffer DV. Designer gene delivery vectors: molecular engineering and evolution of adeno-associated viral vectors for enhanced gene transfer. Pharm Res. 2008;25(3):489–99. https://doi.org/10.1007/s11095-007-9431-0.
230. Takahashi M, Miyoshi H, Verma IM, Gage FH. Rescue from photoreceptor degeneration in the Rd mouse by human immunodeficiency virus vector-mediated gene transfer. J Virol. 1999;73(9):7812–6. https://doi.org/10.1128/JVI.73.9.7812-7816.1999.
231. Koch SF, Tsai YT, Duong JK, Wu WH, Hsu CW, Wu WP, Bonet-Ponce L, Lin CS, Tsang SH. Halting progressive neurodegeneration in advanced retinitis pigmentosa. J Clin Invest. 2015;125(9):3704–13. https://doi.org/10.1172/JCI82462.
232. Tosi J, Sancho-Pelluz J, Davis RJ, Hsu CW, Wolpert KV, Sengillo JD, Lin CS, Tsang SH. Lentivirus-mediated expression of cDNA and ShRNA slows degeneration in retinitis pigmentosa. Exp Biol Med (Maywood). 2011;236(10):1211–7. https://doi.org/10.1258/ebm.2011.011053.
233. Yao J, Jia L, Khan N, Zheng Q-D, Moncrief A, Hauswirth WW, Thompson DA, Zacks DN. Caspase Inhibition with XIAP as an adjunct to AAV vector gene-replacement therapy: improving efficacy and prolonging the treatment window. PLoS ONE. 2012;7(5):e37197.
234. Dias MF, Joo K, Kemp JA, Fialho SL, da Silva Cunha A Jr., Woo SJ, Kwon YJ. Molecular genetics and emerging therapies for retinitis pigmentosa: basic research and clinical perspectives. Prog Retin Eye Res. 2018;63:107–31. https://doi.org/10.1016/j.preteyeres.2017.10.004.
235. Mingozzi F, High KA. Overcoming the host immune response to adeno-associated virus gene delivery vectors: the race between clearance, tolerance, neutralization, and escape. Annual Rev Virol. 2017;4:511–34.
236. Calcedo R, Morizono H, Wang L, McCarter R, He J, Jones D, Batshaw ML, Wilson JM. Adeno-associated virus antibody profiles in newborns, children, and adolescents. Clin Vaccine Immunol. 2011;18(9):1586–8. https://doi.org/10.1128/CVI.05107-11.
237. Li C, Narkbunnam N, Samulski RJ, Asokan A, Hu G, Jacobson LJ, Manco-Johnson MJ, Monahan PE. Joint outcome study I. Neutralizing antibodies against adeno-associated virus examined prospectively in pediatric patients with hemophilia. Gene Ther. 2012;19(3):288–94. https://doi.org/10.1038/gt.2011.90.
238. Gao G-P, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proceedings of the National Academy of Sciences. 2002;99(18):11854-9.
239. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razavi M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, Lessard RR, Sommer JM, Tigges M, Sabatino D, Luk A, Jiang H, Mingozzi F, Couto L, Ertl HC, High KA, Kay MA. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med. 2006;12(3):342–7. https://doi.org/10.1038/nm1358.
240. Jiang H, Couto LB, Patarroyo-White S, Liu T, Nagy D, Vargas JA, Zhou S, Scallan CD, Sommer J, Vijay S, Mingozzi F, High KA, Pierce GF. Effects of transient immunosuppression on adenoassociated, virus-mediated, liver-directed gene transfer in rhesus macaques and implications for human gene therapy. Blood. 2006;108(10):3321–8. https://doi.org/10.1182/blood-2006-04-017913.
241. Scallan CD, Jiang H, Liu T, Patarroyo-White S, Sommer JM, Zhou S, Couto LB, Pierce GF. Human Immunoglobulin inhibits liver transduction by AAV vectors at low AAV2 neutralizing titers in SCID mice. Blood. 2006;107(5):1810–7.
242. Meliani A, Leborgne C, Triffault S, Jeanson-Leh L, Veron P, Mingozzi F. Determination of anti-adeno-associated virus vector neutralizing antibody titer with an in vitro reporter system. Hum Gene Ther Methods. 2015;26(2):45–53. https://doi.org/10.1089/hgtb.2015.037.
243. Manno CS, Chew AJ, Hutchison S, Larson PJ, Herzog RW, Arruda VR, Tai SJ, Ragni MV, Thompson A, Ozelo M, Couto LB, Leonard DG, Johnson FA, McClelland A, Scallan C, Skarsgard E, Flake AW, Kay MA, High KA, Glader B. AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B. Blood. 2003;101(8):2963–72. https://doi.org/10.1182/blood-2002-10-3296.
244. Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. Nat Rev Genet. 2020;21(4):255–72. https://doi.org/10.1038/s41576-019-0205-4.
245. Wu Z, Sun J, Zhang T, Yin C, Yin F, Van Dyke T, Samulski RJ, Monahan PE. Optimization of self-complementary AAV vectors for liver-directed expression results in sustained correction of hemophilia B at low vector dose. Mol Ther. 2008;16(2):280–9.
246. Rangarajan S, Walsh L, Lester W, Perry D, Madan B, Laffan M, Yu H, Vettermann C, Pierce GF, Wong WY, Pasi KJ. AAV5-Factor VIII gene transfer in severe hemophilia A. N Engl J Med. 2017;377(26):2519–30. https://doi.org/10.1056/NEJMoa1708483.
247. Chuah MK, Petrus I, De Bleser P, Le Guiner C, Gernoux G, Adjali O, Nair N, Willems J, Evens H, Rincon MY, Matrai J, Di Matteo M, Samara-Kuko E, Yan B, Acosta-Sanchez A, Meliani A, Cherel G, Blouin V, Christophe O, Moullier P, Mingozzi F, VandenDriessche T. Liver-specific transcriptional modules identified by genome-wide in Silico analysis enable efficient gene therapy in mice and non-human primates. Mol Ther. 2014;22(9):1605–13. https://doi.org/10.1038/mt.2014.114.
248. Rincon MY, Sarcar S, Danso-Abeam D, Keyaerts M, Matrai J, Samara-Kuko E, Acosta-Sanchez A, Athanasopoulos T, Dickson G, Lahoutte T. Genome-wide computational analysis reveals cardiomyocyte-specific transcriptional cis-regulatory motifs that enable efficient cardiac gene therapy. Mol Ther. 2015;23(1):43–52.
249. Brown HC, Zakas PM, George SN, Parker ET, Spencer HT, Doering CB. Target-Cell-Directed bioengineering approaches for gene therapy of hemophilia A. Mol Ther Methods Clin Dev. 2018;9:57–69. https://doi.org/10.1016/j.omtm.2018.01.004.
250. Bowles DE, McPhee SW, Li C, Gray SJ, Samulski JJ, Camp AS, Li J, Wang B, Monahan PE, Rabinowitz JE, Grieger JC, Govindasamy L, Agbandje-McKenna M, Xiao X, Samulski RJ. Phase 1 gene therapy for Duchenne muscular dystrophy using a translational optimized AAV vector. Mol Ther. 2012;20(2):443–55. https://doi.org/10.1038/mt.2011.237.
251. Hastie E, Samulski RJ. Adeno-associated virus at 50: a golden anniversary of discovery, research, and gene therapy success—a personal perspective. Hum Gene Ther. 2015;26(5):257–65.

252. Hirsch ML, Wolf SJ, Samulski RJ. Delivering Transgenic DNA exceeding the carrying capacity of AAV vectors. Methods Mol Biol. 2016;1382:21–39. https://doi.org/10.1007/978-1-4939-3271-9_2.
253. Koo T, Popplewell L, Athanasopoulos T, Dickson G. Triple trans-splicing adeno-associated virus vectors capable of transferring the coding sequence for full-length dystrophin protein into dystrophic mice. Hum Gene Ther. 2014;25(2):98–108.
254. Hirsch ML, Li C, Bellon I, Yin C, Chavala S, Pryadkina M, Richard I, Samulski RJ. Oversized AAV transductifon is mediated via a DNA-PKcs-independent, Rad51C-dependent repair pathway. Mol Ther. 2013;21(12):2205–16.
255. Fakhiri J, Schneider MA, Puschhof J, Stanifer M, Schildgen V, Holderbach S, Voss Y, El Andari J, Schildgen O, Boulant S, Meister M, Clevers H, Yan Z, Qiu J, Grimm D. Novel chimeric gene therapy vectors based on Adeno-Associated virus and four different mammalian bocaviruses. Mol Ther Methods Clin Dev. 2019;12:202–22. https://doi.org/10.1016/j.omtm.2019.01.003.
256. Tornabene P, Trapani I, Minopoli R, Centrulo M, Lupo M, de Simone S, Tiberi P, Dell'Aquila F, Marrocco E, Iodice C, Iuliano A, Gesualdo C, Rossi S, Giaquinto L, Albert S, Hoyng CB, Polishchuk E, Cremers FPM, Surace EM, Simonelli F, De Matteis MA, Polishchuk R, Auricchio A. Intein-mediated protein trans-splicing expands adeno-associated virus transfer capacity in the retina. Sci Transl Med. 2019;11(492):eaav. https://doi.org/10.1126/scitranslmed.aav4523.
257. Lundstrom K. Viral vectors in gene therapy. Diseases. 2018;6(2):42. https://doi.org/10.3390/diseases6020042.

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