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Review Article

Gene therapy in hereditary retinal dystrophy

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Abstract

Hereditary retinal dystrophies (HRDs), such as retinitis pigmentosa, Leber's congenital amaurosis (LCA), Usher syndrome, and retinoschisis, are a group of genetic retinal disorders exhibiting both genetic and phenotypic heterogeneity. Symptoms include progressive retinal degeneration and constricted visual field. Some patients will be legal or completely blind. Advanced sequencing technologies improve the genetic diagnosis of HRD and lead to a new era of research into gene-targeted therapies. Following the first Food and Drug Administration approval of gene augmentation therapy for LCA caused by *RPE65* mutations, multiple clinical trials are currently underway applying different techniques. In this review, we provide an overview of gene therapy for HRD and emphasize four distinct approaches to gene-targeted therapy that have the potential to slow or even reverse retinal degeneration: (1) viral vector-based and nonviral gene delivery, (2) RNA-based antisense oligonucleotide, (3) genome editing by the Clustered Regularly Interspaced Short Palindromic Repeat/cas9 system, and (4) optogenetics gene therapy.

Keywords: *Antisense oligonucleotides, Clustered Regularly Interspaced Short Palindromic Repeat/cas system, Gene therapy, Optogenetics, Retinal dystrophy*

Introduction

Hereditary retinal dystrophies (HRDs), such as retinitis pigmentosa (RP), Leber's congenital amaurosis (LCA), Usher syndrome, choroideremia, retinoschisis, and Stargardt disease, are a group of genetic retinal disorders exhibiting both genetic heterogeneity and phenotypic heterogeneity that remain major obstacles in ophthalmology despite technology that has changed the scope of medicine. To date, more than 280 genes associated with HRD have been reported (Retnet: http://www.sph.uth.tmc.edu/retnet/; provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX, USA). There is still no curative therapy for HRD at present. The dilemma in understanding HRD is the question of how so many different and diverse primary genetic lesions cause the same clinical manifestation that characterizes HRD.

The clinical diagnosis of HRD encompasses different causes and diverse biological pathways with overlapping symptoms and signs and, in such progressive diseases, variations in time/age at presentation. Despite the complexity of retinal dystrophies, substantial progress has recently been made in identifying new HRD genes and developing high-throughput technologies to screen a panel of HRD genes for disease-causing mutations [1-5]. With the advanced application of next-generation sequencing (NGS), it is becoming routine to parallel large gene panels rather than sequential gene-by-gene testing. The identification of disease-causing genes enhances our understanding of genotype–phenotype correlations to provide effective genetic counseling and promote the development of gene-targeted therapeutics.

Gene therapy emerged immediately after the discovery of DNA. However, the treatment was unsuccessful because of limited knowledge of gene expression and the selection of administering genetic material in the 1960s. Currently, gene therapy in various diseases has been initiated [6-9]. The eye is a general focus of gene therapy because it is a small and enclosed organ with unique immunological properties. Furthermore, the noninvasive imaging system can be used for treatment results compared with untreated eyes [10,11]. When the mutant gene associated with HRD was identified, gene therapy was proven effective in clinical development. Gene therapy produces the somatic cells of patients to generate specific therapeutic proteins for the modulation of genetic diseases.

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This review aims to explore the four areas of research that have enabled the development of viral vector-based and nonviral gene delivery, RNA-based antisense oligonucleotide, genomic editing, and optogenetic approaches for the treatment of HRD. This work provides the progress achieved in the field, which has led to multiple ongoing clinical trials and the limitations that have been addressed for the long-term efficacy and safety evaluation of these treatments.

Viral vector-based and nonviral gene delivery

Gene therapy emerged immediately after the discovery of DNA. However, the treatment was unsuccessful due to limited knowledge of gene expression and the selection of administering genetic material in the 1960s. Currently, gene therapy in various diseases has been initiated [6-9]. The eye is a general focus of gene therapy because it is a small and enclosed organ with unique immunological properties. Furthermore, the noninvasive imaging system can be used for treatment results compared with untreated eyes [10,11]. When the mutant gene associated with HRD was identified, gene therapy was proven in clinical development. Gene therapy produces the somatic cells of patients to generate specific therapeutic proteins for the modulation of genetic diseases. Advances in gene delivery development currently include viral gene therapy systems and nonviral gene delivery systems. Virus-dependent vectors were first identified in the 1980s [12]. The virus infects host cells and inserts DNA fragments into the host genome or by nonintegrating stable transduction [13], replicating the genetic materials and producing protein. Viruses applied for gene therapeutic systems include retrovirus, lentivirus, adenovirus, adeno-associated viruses (AAV), and herpes simplex viruses. Lentiviruses were used to transfect host cells by packaging three cotransfection plasmids, a packaging plasmid, a transfer plasmid, and an envelope plasmid. The target gene is expressed by the cell-specific promoter from lentivirus infection. AAV is the most actively targeted gene therapy vehicle. It has been reported that AAV showed effectiveness and safety in clinical trials without any symptoms interacting with AAV in human diseases [14]. The modification of AAV eliminated the viral-coding DNA, containing two viral genome *cis* packaging signals and packaging the target gene in the vector. AAV can explore target genes in host cells [15]. Different target genes for HRD have been developed with AAV subtype vectors. The clinical trial *PRE65* gene viral gene therapy in retinal dystrophy was approved by the first Food and Drug Administration (FDA) [16]. Afterward, many specified genes associated with HRD were delivered for treating RP [17-19], Bietti's crystalline dystrophy (NCT No. 04722107), choroideremia [20,21], LCA [22], X-linked retinoschisis [23], and Stargardt diseases [20] in clinical trials. The current gene therapy clinical trials for HRD are listed in Table 1. Multiple mechanisms have been reported related to strategies for gene therapy. Genes have loss-of-function mutations, and the AAV system with the target of genes expressed the functional protein, which rescued the enzyme deficiency in diseases. The goal of treatment for genes with gain-of-function mutation is to inhibit the expression of mutant proteins using an allele-specific AAV system with the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) or RNAi and express the ratio of wild-type proteins by AAV delivery. It has been reported that gene therapy of the *RHO* gene involves knocking down one mutated copy of *RHO* using the allele-specific inhibition CRISPR system or suppressing endogenous protein expression by the RNAi pathway. The functional *RHO* gene is expressed by AAV delivery [24,25]. However, the limitation of the viral-dependent gene of AAV therapy is <5 kb of packaged gene size in the vector. The gene delivery of large genes is restricted to therapy. Neutralized antibodies are activated from AAV, decreasing the efficacy of gene therapy [26]. Although lentivirus transduces nondividing cells and packages more than 10 kb of gene size, insertional mutagenesis is a significant disadvantage in human gene therapy [27]. Nonviral gene therapy is simple to use, has large-scale production, and lacks inflammation in humans. Multiple nonviral genes have been developed for different clinical settings, including target tissue injection by transfecting or packaging naked DNA with a variety of particles [28]. Naked DNA can be delivered with vehicles, including liposomes, biodegradable polymers, and nanoparticles [29]. However, the different molecular weights or isoforms of polymers reveal endurance with different transfection efficiencies and toxicities. In the future, the interaction between structure and function may induce the development of novel, efficient gene therapies that lack toxicity.

RNA-based antisense oligonucleotide

RNA-based antigen oligonucleotides (AOs) bind to endogenous RNA to modulate RNA expression [30,31]. The first AO, which is approved by the FDA, is delivered for the treatment of cytomegalovirus retinitis [32]. The mechanisms of RNA-based AO therapeutics are dependent on the endogenous microRNA (miRNA) pathway. miRNAs are transcribed from protein noncoding regains by RNA polymerase II. After production by RNase III and Drosha protein, the RNA hairpin formation occurred as pre-miRNA. Pre-miRNAs pass through the nucleus and are modified by Dicer, cleavage to a miRNA duplex. One strand of the miRNA binds to Argonaute protein (Ago) to form the RNA-induced silencing complex (RISC), and the strand of miRNA is degraded. The RISC complex guides endogenous mRNAs and miRNAs to bind to target mRNAs, causing deadenylation and degradation of the transcript [33]. Because of the extensive modulation of gene regulation, miRNA expression is controlled by development and space in different states. Therefore, miRNA expression plays an important role in the progression of multiple diseases [34,35]. The RNA-based AO can bind to target mRNA and pre-mRNA, blocking protein translation or guiding RNase H to bind to target transcription and degradation of RNA [36]. RNA-based AOs can easily interact with host cells, but this drug can be degraded by nucleases, and this RNA base is negatively charged and induces inflammation. The RNA-based AO is packaged with polyethylene glycol, lipids, polymers, and viral vectors [37]. Currently, the RNA-based AO is used to treat retinal disorders as AntimiR-155 (MRG-107) in a preclinical trial [38].

Table 1: Current gene therapy clinical trials for hereditary retinal dystrophy (update to May 22, 2022)

Disease	Gene	Vector	Delivery	Gene therapy strategy	Phase	Sponsor	NCT number
RP	*RLBP1*	AAV8 (CPK850)	Subretinal	Augmentation	1/2	Novartis pharmaceuticals	03374657
	PDE6A	AAV8	Subretinal	Augmentation	1/2	STZ eyetrial	04611503
	NR2E3	AAV5 (OCU400)	Subretinal	Augmentation	1/2	Ocugen	05203939
	Channelrhodopsin (ChronosFP)	AAV2 (BS01)	Subretinal	Optogenetic	1/2	Bionic Sight LLC	04278131
	PDE6B	AAV2/5	Subretinal	Augmentation	1/2	Horama S.A	03328130
	ChR2	AAV2 (RST-001)	Intravitreal	Optogenetic	1/2	Allergan	02556736
	RPGR	AAV (codon-optimized human *RPGR*, 4D-125)	Intravitreal	Augmentation	1/2	4D molecular therapeutics	04517149
	RPGR	AAV5	Subretinal	Augmentation	3	MeiraGTx UK II Ltd.	04671433
	Channelrhodopsin ChrimsonR-tdTomato	AAV2.7m8 (GS030)	Intravitreal	Optogenetic	1/2	GenSight Biologics	03326336
	RPGR	AAV2tYF-GRK-1 (AGTC-501)	Subretinal	Augmentation	2/3	Applied Genetic Technologies Corp	04850118
	Multi-Characteristic Opsin	AAV2 (vMCO-010)	Intravitreal	Optogenetics	2	Nanoscope Therapeutics Inc.	04945772
	Rhodopsin (P23H mutation)	Antisense oligonucleotide (QR-1123)	Intravitreal	Antisense oligonucleotide	1/2	ProQR Therapeutics	04123626
	USH2A	Antisense oligonucleotide (QR-421a)	Intravitreal	*USH2A* exon13 skipping	2/3	ProQR Therapeutics	05158296
	MERTK	AAV2	Subretinal	Augmentation	1	King Khaled Eye Specialist Hospital	01482195
Bietti's crystalline dystrophy	*CYP4V2*	AAV2/8	Subretinal	Augmentation	1	Beijing Tongren Hospital	04722107
Choroideremia	*CHM/REP1*	AAV2	Subretinal	Augmentation	2	Byron Lam University of Miami	02553135
	CHM/REP1	AAV2	Subretinal	Augmentation	1/2	Ian M. MacDonald	02077361
	CHM	AAV capsid variant (4D-R100)	Intravitreal	Condon-optimized Augmentation	1	4D molecular therapeutics	04483440
	CHM/REP1	AAV2 (BIIB111)	Subretinal	Augmentation	3	Biogen	03496012
	CHM/RPE1	AAV2	Subretinal	Augmentation	2	STZ eyetrial	02671539
	CHM/RPE1	AAV2	Subretinal	Augmentation	2	University of Oxford	02407678
	CHM	AAV2	Subretinal	Augmentation	1/2	Spark Therapeutics	02341807
LCA	*CEP290* (p.Cys998X mutation)	RNA antisense oligonucleotide (QR-110)	Intravitreal	Correct the splicing defect	2/3	ProQR Therapeutics	03140969
	CEP290 (c. 2991+1655A>G in intron 26 (IVS26))	CRISPR/Cas9 (EDIT-101)	Subretinal	Gene editing	1/2	Editas Medicine, Inc.	03872479
	RPE65	AAV2	Subretinal	Augmentation	3	Novartis pharmaceuticals	04516369
	RPE65	AAV2 (rAAV2-CBSB-hRPE65)	Subretinal	Augmentation	1	University of pennsylvania	00481546
	RPE65	AAV2 (AAV2-hRPE65v2, voretigene neparvovec-rzyl)	Subretinal	Augmentation	3	Spark therapeutics	00999609
	GUCY2D	AAV5	Subretinal	Augmentation	1/2	Atsena Therapeutics Inc.	03920007
X-linked retinoschisis	*RS1*	AAV2	Subretinal	Augmentation	1/2	Applied Genetic Technologies, Corp.	02416622
	RS1	AAV8	Subretinal	Augmentation	1/2	National Eye Institute	02317887
Stargardt diseases	*ABCA4*	LV (SAR422459)	Subretinal	Augmentation	1/2	Sanofi	01367444

ChR2: *Channelrhodopsin-2*, RPGR: Retinitis pigmentosa GTPase regulator, LCA: Leber congenital amaurosis, RP: Retinitis pigmentosa, AAV: Adeno-associated viruses, CRISPR: Clustered regularly interspaced short palindromic repeat

However, miRNA regulation in humans is a complex network with few confirmed targets. Furthermore, to increase the treatment effect, RNA-based AOs require higher doses to increase effectiveness, which may change drug expression and induce the immune response [39]. In the future, developing the coadministration of standard therapy with RNA-based AOs may emerge as a benefit for HRD.

Genome editing by the clustered regularly interspaced short palindromic repeat/Cas9 system

HRDs are the disease most studied in gene therapy because HRD is still not an effective treatment strategy. The allele-specific ablation rescue via CRISPR and

CRISPR-associated nuclease (Cas) 9 system (CRISPR/Cas9) might provide an effective implement for inherited disease in patients [40]. The CRISPR system was first observed in strains of *E. coli* in the 1980s [41]. The CRISPR system is the immune system of bacteria and archaea against foreign genetic material infection. Short regularly spaced repeats were discovered in the CRISPR array of DNA genetic material. After foreign DNA infection, the foreign DNA fragments are consolidated in the CRISPR array, and this system contains the conserved repeats and target-specific spacers (protospacers) of genetic elements. Subsequently, the bacteria transcribe the CRISPR RNAs (crRNAs) from this array. The crRNAs cooperate with Cas protein to activate the surveillance complex. This complex recognizes foreign DNA fragments, and crRNAs directly target the protospacer adjacent motif of downstream sequencing. Following crRNA guide binding, Cas cleaves the targeted sequence to protect against foreign infection [42-44]. Current Studies have reported that the modified CRISPR/Cas9 system may mediate genomic editing in mammalian cells to correct the disease-causing mutation. Several studies have been conducted treating animal models of HRDs using the CRISPR/Cas9 system. The genetic mutation containing five stop codons generated at position 334 (S334ter) was found in the autosomal dominant RP, leading to the loss of function of rhodopsin and causing blindness. This mutation can be corrected in the rodent animal model by subretinal injection with the Cas9/gRNA^{TRGT} system [45]. In similar studies, loss of neural retina leucine zipper (NRL) protein function in rod photoreceptors causes RP, as previously reported. In a mouse animal model, the AAV 8 vector delivered SpCas9, and sgRNA was subretinally injected and recused the visual function and delayed *Nrl* disruption in photoreceptors [46]. Mutations in the RP GTPase regulator (RPGR) gene also cause RP of HRD. Subretinal injections of AAV-sgRNA and-SpCas9 expression plasmids in mouse eyes restored the reading frame of the *RPGR* gene to repair the protein in retina [47]. In a patient-specific induced pluripotent stem cell (iPSC) study, iPSCs were infected with an AAV vector containing sgRNA and Cas9 and delivered to CEP290-and RHO-patient-specific iPSCs to prevent translation of the mutant disease-causing gene LCA [48]. The CRISPR/Cas9 system provides a strategy to correct disease, which causes variants of genomic location and inheritance patterns. However, the therapeutic effects of CRISPR/Cas9 have been challenging *in vivo*, and the system may cause nonspecific editing at other untargeted genetic loci called off-target mutations. This phenomenon could induce pathogenic consequences or might be silent in the genome. In addition, this system needs to be confirmed to have more efficient therapeutic efficacy in disease. The mechanism of the NHEJ and HDR repair pathways should be understood after the CRISPR/Cas9 system [49,50].

Optogenetics gene therapy

Optogenetics is the integration of optics and genetics to perform the loss-of-function or gain-of-function in the targeted protein of living tissue [51-53]. The first bacteriorhodopsin was confirmed as the photoactivated ion pump in the 1970s [54]. Afterward, different bacteriorhodopsins were published in the ensuing decades, including transmembrane ion pumps and channels. These microbial opsins can cross the membrane and respond to photons [55,56]. Currently, optogenetics can be used in a neural system without mutation and with specific restoration of circuits in neurological diseases [57]. HRDs are neurodegenerative eye diseases. Because the eye is transparent and photosensitive to retinal neural layers, surgery is less invasive. Optogenetics may restore visual function by targeting opsin to the retina as the success of the strategy [58]. The treatment strategy to restore visual function is to package the visual opsin sequence with a retinal-specific promotor in AAV intraocularly injected into a patient. channelrhodopsin was expressed on the retinal ganglion layer and responded to light stimulation. A previous study confirmed that in rodents intravitreally injected with an AAV-channelrhodopsin-2 (ChR2) construct, ChR2 can stabilize expression in the retina and rescue visual function [59]. In a nonhuman primate study, primates (*Macaca fascicularis*) were intravitally injected with an AAV2 vector with ChR2 to stimulate retinal activation after long-term transfection [60]. Recently, optogenetics was used to treat a blind patient in a phase 1/2a clinical trial. After intravitreal injection with AAV vector expressing the fusion protein ChrimsoR-tdTomato, the light (590nm) was stimulated from googles, the patient performed functional visual tests and recorded the EEG data. The tests described that the patient perceived the location of objects with light-stimulated goggles. The results demonstrated partial recovery in visual function after 14 months of optogenetic therapy [57]. Optogenetics is a powerful therapy for visual restoration without complex surgery with signal intravitreal injection of the AAV-ChR2 construct. In addition, the safety of AAV delivery is well established in human genetic treatment. However, identifying and organizing target neural circuits in the mammalian retina is a limitation for therapy. The cause of HRD is progressive disease. Dysfunction of photoreceptor cells destroys the synaptic connection to bipolar cells; photoreceptor cell death leads to the decimation of bipolar cell dendrites and separates the neural retina from the retinal pigment epithelium (RPE) and choroid; glial cell promotor remodeling in the retina and RPE cells invade the neural retina, leading to the formation of scars in the retina [61]. The chaotic reprogramming of the retinal layer using an optogenetics system is still challenging [62].

Conclusions

HRD, a major public health problem, is currently not a curative treatment. Applying NGS to these HRD families will identify disease-causing genes and mutations and contribute to a better understanding of the relationship between mutations and clinical outcomes. Furthermore, knowing the underlying cause of disease, the genotype, is an essential requirement for enrolling in most clinical trials. Adeno-associated viral vector (AAV)-targeted gene transfer offers several advantages as a gene delivery vehicle. Currently, it is a feasible and dominant approach for the treatment of HRD patients. The goals of these initial studies were primarily to test the safety and efficacy of viral gene therapy, and these trials demonstrated acceptable immunologic changes with no serious adverse effects or evidence of efficacy. Recent developments

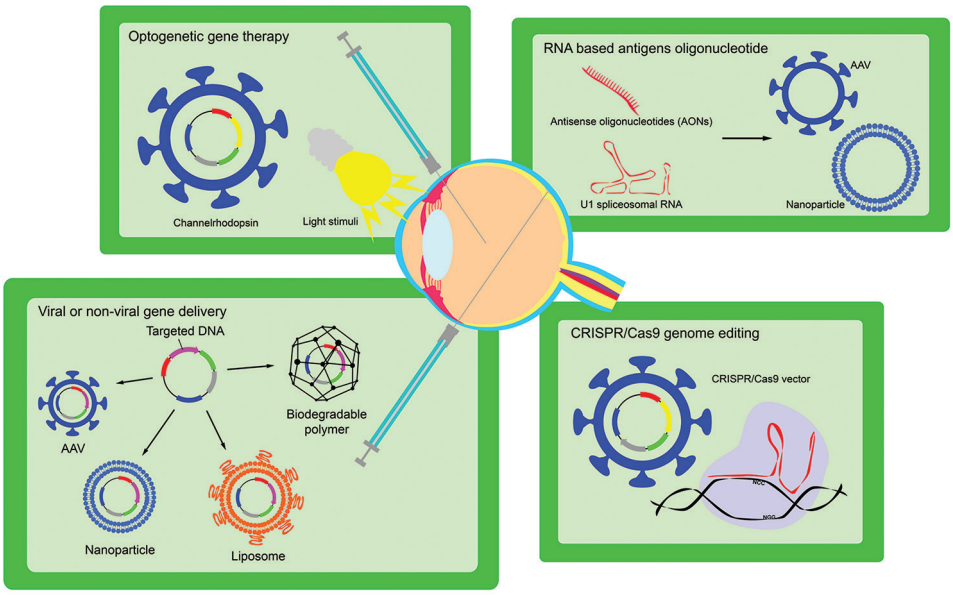

Figure 1: Summary diagram of gene-targeted therapies for hereditary retinal dystrophies. Recent developments in various gene-targeted therapies, including viral or nonviral based gene delivery, optogenetics, genomic editing, or RNA-based therapeutics, could offer potential treatments for HRD patients. HRD: Hereditary retinal dystrophies

in various gene-targeted therapies, including optogenetics, genomic editing, and RNA-based therapeutics [Figure 1], could offer the possibility of finding cures for HRD patients. These invaluable data will translate the beneficial effects on future clinical applications.

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Conflicts of interest

There are no conflicts of interest.

References

1. Carrigan M, Duignan E, Malone CP, Stephenson K, Saad T, McDermott C, et al. Panel-based population next-generation sequencing for inherited retinal degenerations. Sci Rep 2016;6:33248.
2. Ge Z, Bowles K, Goetz K, Scholl HP, Wang F, Wang X, et al. NGS-based Molecular diagnosis of 105 eyeGENE(®) probands with Retinitis Pigmentosa. Sci Rep 2015;5:18287.
3. Bravo-Gil N, Méndez-Vidal C, Romero-Pérez L, González-del Pozo M, Rodríguez-de la Rúa E, Dopazo J, et al. Improving the management of Inherited Retinal Dystrophies by targeted sequencing of a population-specific gene panel. Sci Rep 2016;6:23910.
4. Birtel J, Gliem M, Mangold E, Müller PL, Holz FG, Neuhaus C, et al. Next-generation sequencing identifies unexpected genotype-phenotype correlations in patients with retinitis pigmentosa. PLoS One 2018;13:e0207958.
5. Salmaninejad A, Motaee J, Farjami M, Alimardani M, Esmaeilie A, Pasdar A. Next-generation sequencing and its application in diagnosis of retinitis pigmentosa. Ophthalmic Genet 2019;40:393-402.
6. Berger W, Kloeckener-Gruissem B, Neidhardt J. The molecular basis of human retinal and vitreoretinal diseases. Prog Retin Eye Res 2010;29:335-75.
7. Dalkara D, Sahel JA. Gene therapy for inherited retinal degenerations. C R Biol 2014;337:185-92.
8. Sahel JA, Marazova K, Audo I. Clinical characteristics and current therapies for inherited retinal degenerations. Cold Spring Harb Perspect Med 2014;5:a017111.
9. Trapani I, Banfi S, Simonelli F, Surace EM, Auricchio A. Gene therapy of inherited retinal degenerations: Prospects and challenges. Hum Gene Ther 2015;26:193-200.
10. Trapani I, Puppo A, Auricchio A. Vector platforms for gene therapy of inherited retinopathies. Prog Retin Eye Res 2014;43:108-28.
11. Sahel JA, Roska B. Gene therapy for blindness. Annu Rev Neurosci 2013;36:467-88.
12. Moss B, Smith GL, Gerin JL, Purcell RH. Live recombinant vaccinia virus protects chimpanzees against hepatitis B. Nature 1984;311:67-9.
13. Yáñez-Muñoz RJ, Balaggan KS, MacNeil A, Howe SJ, Schmidt M, Smith AJ, et al. Effective gene therapy with nonintegrating lentiviral vectors. Nat Med 2006;12:348-53.
14. Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-associated virus (AAV) as a vector for gene therapy. BioDrugs 2017;31:317-34.
15. Nayerossadat N, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. Adv Biomed Res 2012;1:27.
16. Bennett J, Wellman J, Marshall KA, McCague S, Ashtari M, DiStefano-Pappas J, et al. Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by RPE65 mutations: A follow-on phase 1 trial. Lancet 2016;388:661-72.
17. Parinot C, Nandrot EF. A comprehensive review of mutations in the MERTK proto-oncogene. Adv Exp Med Biol 2016;854:259-65.
18. Palmowski-Wolfe A, Stingl K, Habibi I, Schorderet D, Tran HV. Novel PDE6B mutation presenting with retinitis pigmentosa – A case series of three patients. Klin Monatsbl Augenheilkd 2019;236:562-7.
19. Dulla K, Slijkerman R, van Diepen HC, Albert S, Dona M, Beumer W, et al. Antisense oligonucleotide-based treatment of retinitis pigmentosa caused by USH2A exon 13 mutations. Mol Ther 2021;29:2441-55.
20. Davis JL. The blunt end: Surgical challenges of gene therapy for inherited retinal diseases. Am J Ophthalmol 2018;196:xxv-xxix.
21. Dimopoulos IS, Hoang SC, Radziwon A, Binczyk NM, Seabra MC, MacLaren RE, et al. Two-year results After AAV2-mediated gene therapy for choroideremia: The Alberta experience. Am J Ophthalmol 2018;193:130-42.
22. Russell S, Bennett J, Wellman JA, Chung DC, Yu ZF, Tillman A, et al.

Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: A randomised, controlled, open-label, phase 3 trial. Lancet 2017;390:849-60.

23. Cehajic-Kapetanovic J, Xue K, Martinez-Fernandez de la Camara C, Nanda A, Davies A, Wood LJ, et al. Initial results from a first-in-human gene therapy trial on X-linked retinitis pigmentosa caused by mutations in RPGR. Nat Med 2020;26:354-9.
24. Murray SF, Jazayeri A, Matthes MT, Yasumura D, Yang H, Peralta R, et al. Allele-specific inhibition of rhodopsin with an antisense oligonucleotide slows photoreceptor cell degeneration. Invest Ophthalmol Vis Sci 2015;56:6362-75.
25. Meng D, Ragi SD, Tsang SH. Therapy in rhodopsin-mediated autosomal dominant retinitis pigmentosa. Mol Ther 2020;28:2139-49.
26. Colella P, Ronzitti G, Mingozzi F. Emerging issues in AAV-mediated *in vivo* gene therapy. Mol Ther Methods Clin Dev 2018;8:87-104.
27. Milone MC, O'Doherty U. Clinical use of lentiviral vectors. Leukemia 2018;32:1529-41.
28. Li S, Huang L. Nonviral gene therapy: Promises and challenges. Gene Ther 2000;7:31-4.
29. Koirala A, Conley SM, Naash MI. A review of therapeutic prospects of non-viral gene therapy in the retinal pigment epithelium. Biomaterials 2013;34:7158-67.
30. Le BT, Raguraman P, Kosbar TR, Fletcher S, Wilton SD, Veedu RN. Antisense oligonucleotides targeting angiogenic factors as potential cancer therapeutics. Mol Ther Nucleic Acids 2019;14:142-57.
31. Dias N, Stein CA. Antisense oligonucleotides: Basic concepts and mechanisms. Mol Cancer Ther 2002;1:347-55.
32. Roehr B. Fomivirsen approved for CMV retinitis. J Int Assoc Physicians AIDS Care 1998;4:14-6.
33. Bajan S, Hutvagner G. RNA-based therapeutics: From antisense oligonucleotides to miRNAs. Cells 2020;9:E137.
34. Bajan S, Hutvagner G. Regulation of miRNA processing and miRNA mediated gene repression in cancer. Microrna 2014;3:10-7.
35. Gorabi AM, Bianconi V, Pirro M, Banach M, Sahebkar A. Regulation of cardiac stem cells by microRNAs: State-of-the-art. Biomed Pharmacother 2019;120:109447.
36. Bennett CF, Baker BF, Pham N, Swayze E, Geary RS. Pharmacology of antisense drugs. Annu Rev Pharmacol Toxicol 2017;57:81-105.
37. Kaczmarek JC, Kowalski PS, Anderson DG. Advances in the delivery of RNA therapeutics: From concept to clinical reality. Genome Med 2017;9:60.
38. Chu-Tan JA, Natoli R. The potential for microRNA-based therapeutics in retinal disorders. Ann Transl Med 2020;8:419.
39. Hu W, Tan C, He Y, Zhang G, Xu Y, Tang J. Functional miRNAs in breast cancer drug resistance. Onco Targets Ther 2018;11:1529-41.
40. Wu W, Tang L, D'Amore PA, Lei H. Application of CRISPR-Cas9 in eye disease. Exp Eye Res 2017;161:116-23.
41. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. J Bacteriol 1987;169:5429-33.
42. Leenay RT, Beisel CL. Deciphering, communicating, and engineering the CRISPR PAM. J Mol Biol 2017;429:177-91.
43. Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol Evol 2005;60:174-82.
44. Pourcel C, Salvignol G, Vergnaud G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology (Reading) 2005;151:653-63.
45. Bakondi B, Lv W, Lu B, Jones MK, Tsai Y, Kim KJ, et al. *In vivo* CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa. Mol Ther 2016;24:556-63.
46. Yu W, Mookherjee S, Chaitankar V, Hiriyanna S, Kim JW, Brooks M, et al. Nrl knockdown by AAV-delivered CRISPR/Cas9 prevents retinal degeneration in mice. Nat Commun 2017;8:14716.
47. Gumerson JD, Alsufyani A, Yu W, Lei J, Sun X, Dong L, et al. Restoration of RPGR expression *in vivo* using CRISPR/Cas9 gene editing. Gene Ther 2022;29:81-93.
48. Burnight ER, Gupta M, Wiley LA, Anfinson KR, Tran A, Triboulet R, et al. Using CRISPR-Cas9 to generate gene-corrected autologous iPSCs for the treatment of inherited retinal degeneration. Mol Ther 2017;25:1999-2013.
49. Li L, Hu S, Chen X. Non-viral delivery systems for CRISPR/Cas9-based genome editing: Challenges and opportunities. Biomaterials 2018;171:207-18.
50. Chu VT, Weber T, Wefers B, Wurst W, Sander S, Rajewsky K, et al. Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. Nat Biotechnol 2015;33:543-8.
51. Yizhar O, Fenno LE, Davidson TJ, Mogri M, Deisseroth K. Optogenetics in neural systems. Neuron 2011;71:9-34.
52. Deisseroth K, Feng G, Majewska AK, Miesenböck G, Ting A, Schnitzer MJ. Next-generation optical technologies for illuminating genetically targeted brain circuits. J Neurosci 2006;26:10380-6.
53. Deisseroth K. Optogenetics. Nat Methods 2011;8:26-9.
54. Oesterhelt D, Stoeckenius W. Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. Nat New Biol 1971;233:149-52.
55. Ritter E, Stehfest K, Berndt A, Hegemann P, Bartl FJ. Monitoring light-induced structural changes of Channelrhodopsin-2 by UV-visible and Fourier transform infrared spectroscopy. J Biol Chem 2008;283:35033-41.
56. Zhang F, Prigge M, Beyrière F, Tsunoda SP, Mattis J, Yizhar O, et al. Red-shifted optogenetic excitation: A tool for fast neural control derived from Volvox carteri. Nat Neurosci 2008;11:631-3.
57. Sahel JA, Boulanger-Scemama E, Pagot C, Arleo A, Galluppi F, Martel JN, et al. Partial recovery of visual function in a blind patient after optogenetic therapy. Nat Med 2021;27:1223-9.
58. Henriksen BS, Marc RE, Bernstein PS. Optogenetics for retinal disorders. J Ophthalmic Vis Res 2014;9:374-82.
59. Bi A, Cui J, Ma YP, Olshevskaya E, Pu M, Dizhoor AM, et al. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. Neuron 2006;50:23-33.
60. Chaffiol A, Provansal M, Joffrois C, Blaize K, Labernede G, Goulet R, et al. *In vivo* optogenetic stimulation of the primate retina activates the visual cortex after long-term transduction. Mol Ther Methods Clin Dev 2022;24:1-10.
61. Marc RE, Jones BW, Anderson JR, Kinard K, Marshak DW, Wilson JH, et al. Neural reprogramming in retinal degeneration. Invest Ophthalmol Vis Sci 2007;48:3364-71.
62. Barrett JM, Berlinguer-Palmini R, Degenaar P. Optogenetic approaches to retinal prosthesis. Vis Neurosci 2014;31:345-54.