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Attenuation of Inherited and Acquired Retinal Degeneration Progression with Gene-based Techniques

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Abstract

Inherited retinal dystrophies cause progressive vision loss and are major contributors to blindness worldwide. Advances in gene therapy have brought molecular approaches into the realm of clinical trial for these incurable illnesses. Select phase I, II and III trials are complete and provide some promise in terms of functional outcomes and safety; although questions do remain over the durability of their effects and the prevalence of inflammatory reactions. This article reviews gene therapy as it can be applied to inherited retinal dystrophies, provides an update of results from recent clinical trials, and discusses the future prospects of gene therapy and genome surgery.

I. Introduction:

The retina transmits visual information to the brain, and is thus delicate neural tissue without plasticity [1]. An unfortunate consequence is that cells of the retina affected by inherited dystrophies do not regenerate in humans. For this reason, treatment of retinal degeneration is extremely difficult as photoreceptor death results in irreversible blindness [1, 2]. For approximately 1 in 2000 individuals worldwide, inherited retinal dystrophies (IRDs) cause progressive photoreceptor loss and eventual blindness [3]. The pathophysiology of various

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IRDs are slowly being unraveled over the past few decades—but despite increased knowledge, conventional medicine has not been able to provide cures for these illnesses and management has historically relied on specialized genetic counseling, vitamin supplementation [4], low-vision aids, and other behavioral modifications to maximize the use of any remaining retinal function. Visual prognoses for these patients remain difficult to predict even in cases that are gene- dependent.

Traditional gene therapy approaches may prove amenable to populations of various monogenic retinal dystrophies. The phenotype of visual impairment observed in these diseases arises from diverse genetic mutations that result in dysfunction and death of the light-sensing and signal-transmitting cells found in the retinal tissue of the inner lining of the posterior eye. Gene therapy provides a means of restoring a healthy genome in these diseased cells and, consequently, a hope of returning to their normal function [2]. The appropriate type of gene therapy depends on the inheritance pattern of the disease: whereas recessive conditions and those caused by haploinsufficiency can respond to the addition of wild-type alleles supplied by viral vectors [1,2], dominant or dominant-negative conditions require gene suppression or repair [1, 5].

While alternative vectors for gene therapy such as liposomes, nanoparticles, adenovirus, and lentivirus do exist, AAV is currently considered to be the safest and most effective vector in general [1, 6–9]. There are several serotypes of AAV which have been studied for optimal use in the clinical setting [7, 9–12]. Indeed, optimization is imperative to achieve a therapeutic effect as each serotype of AAV attaches to different receptors leading to preferential entry to various cell types [7, 9, 12, 13]. For the retina, AAV2 has been shown to be effective, and is most commonly used [6, 8–10, 14–17]. While AAV is generally considered to be safe, immune response to AAV is not yet fully understood [10, 18]. In this regard, the eye is perhaps an ideal subject of study; the eye is an anatomically superficial, compartmentalized, and relatively immune-privileged organ, which makes it a suitable target for non-systemic treatment [5, 19, 20]. Still, it should be noted that care must be taken when utilizing viral vectors [21] as the blood-retinal barrier may be compromised in retinal disease [22] potentially leading to inflammation or other host immune response. Still, the eye proves further to be an ideal subject of study as clinicians can non- invasively monitor the eye for treatment response and signs of adverse reactions to such novel treatments [1, 23–25].

A landmark study published in 1996, provided a proof of concept for retinal gene therapy to alter the course of autosomal recessive retinitis pigmentosa in a mouse model[26]. This model possessed homozygous mutations in the gene encoding the rod cGMP phosphodiesterase (PDE). The study described the delivery of murine cDNA for the wild-type β -subunit of PDE (β -PDE) via a replication-defective adenovirus. It reported that *in vivo* subretinal injection of this vector could increase β -PDE transcripts and their activity, delaying photoreceptor death by six weeks. Since this seminal work, gene therapy has been investigated as a treatment modality for many inherited retinal diseases [1, 2]. This article reviews recently completed and ongoing clinical trials of gene therapy and briefly describes the future prospects of clustered regularly interspaced short palindromic repeats (CRISPR)-based genome surgery. While this review provides a brief summary of gene therapy in the retina, we recommend additional reviews if the reader is interested in additional knowledge

on background of gene therapy [27], subretinal injection [28], gene and cell-based therapies for inherited retinal disorders [1, 14], AMD gene therapy [29], CRISPR genome surgery in the retina [5, 30].

II. Clinical Trials of Gene Therapy:

As of August 2018, nine clinical trials of gene therapies for retinal dystrophies have been completed (Table 1) and 30 more are in progress (ClinicalTrials.gov, accessed August 6, 2018) (Table 2). All of the completed trials except one have used AAV as their vector of choice (Table 1). Overall, AAV gene therapy trials seem to have had varied success, though some of their positive effects have been transient [1, 31–38]. Still, a limitation to consider in understanding the results from these gene therapy trials is that in early trials, patients with advanced disease receive treatment and these patients may not have sufficient retinal reserve to see an apparent effect [1]. Although these trials form a basis for gene therapy and personalized medicine, it is worth contextualizing their scope: there are over 250 known loci that cause IRDs and disease manifestation is largely heterogeneous [39]. Only completed IRD gene therapy trial results are discussed in detail in this review. Preliminary results are available for some of the trials listed as “Active, but not recruiting” in Table 2, but are not discussed.

II.a. Leber congenital amaurosis (LCA) gene therapy results:

Leber congenital amaurosis (LCA) is a devastating IRD that causes severe visual loss in childhood [40] and affects approximately 2–3 in 100,000 individuals [41]. While LCA has been linked to more than a dozen disease-causative genes, two have been studied in gene therapy clinical trials: *RPE65* and *CEP290*. Of these, results are only available for completed trials supplementing wild-type *RPE65*. *RPE65* is a retinoid isomerase enzyme which recycles visual pigment in the retinal pigment epithelium (RPE) [42]. *RPE65* mutations manifest with a pigmented retina, nystagmus, amaurotic pupils, and severe visual dysfunction at birth [43]. Completed clinical trials (Table 1) have shown that adeno-associated virus (AAV) vector *RPE65* is safe and effective [16, 32, 37, 44–50]. Of interest, results from the Spark Therapeutics (Philadelphia, PA) trial (NCT00516477) of subretinal injection of LUXTURNTM (voretigene neparvovec-rzyl, AAV2-hRPE65v2–101) showed that younger subjects had greater improvement in vision, suggesting early intervention has greater therapeutic potential [50]. Most notably, LUXTURNTM gained Food and Drug Administration (FDA) approval following completion of a phase III randomized controlled trial (NCT00999609) that demonstrated improved functional vision and no serious adverse events [47]. It is the first gene therapy to be FDA-approved. Still, *RPE65* is only one of many disease-causative genes for LCA, and it is worth noting that subretinal drug injection requires invasive ocular surgery with transient retinal detachment, which carries risk of complication [51]. Moreover, a study by Cideciyan et al. concluded that while AAV2-RPE65 gene therapy in patients with *RPE65*-LCA improves vision in the shortterm, retinal degeneration continues to persist in the long-term [52]. Ongoing surveillance of patients receiving treatment will therefore be followed with interest, as the duration of treatment effect is still worthy of further investigation. In theory, patients may require repeated treatments over time.

II.b. Choroideremia (CHM) gene therapy results:

An X-linked dystrophy, choroideremia (CHM) affects mostly males and causes night blindness and progressive constriction of the visual field. Approximately 1 in 50,000 individuals are affected [53]. The phenotype arises from a mutation in the *CHM* gene, which encodes Rab escort protein-1 (REP-1) [54]. REP-1 is involved in intracellular trafficking and its impairment causes degeneration of the choroid, RPE, and retina, which has low levels of REP-2 compared to the rest of the body, where REP-2 is able to compensate for REP-1 dysfunction. Two phase I/II trials have tested subfoveal injection of an AAV vector carrying wild-type REP-1 [33, 55]. Between these trials, three of twelve patients improved in visual acuity, while most stabilized. Though Dimopoulos et al. observed no improvement in microperimetry sensitivity [55] (NCT02077361), MacLaren et al. reported that five of six patients improved on dark-adapted microperimetry and additionally noted that the patient that did not improve had received a reduced dose of the vector [33] (NCT01461213). A few notable injection-related complications and adverse events did occur: one patient experienced an intraretinal inflammatory reaction at the site of injection as perioperative steroids were tapered. This caused a severe decline in visual acuity associated with loss of the outer retinal layers. It is worth noting that, as with LCA, no proven treatment currently exists for CHM and a certain level of risk may be tolerated.

II.c. Leber Hereditary Optic Neuropathy (LHON) gene therapy results:

Leber hereditary optic neuropathy (LHON) is an inherited optic disc atrophy which leads to bilateral visual loss, caused by pathogenic variants in mitochondrial DNA (mtDNA) [56]. Affecting approximately 1 in 45,000 individuals in Europe, LHON is considered to be the most prevalent mitochondrial disease [57]. While LHON affects retinal ganglion cells (RGCs) in particular, the mechanism of degeneration is still not fully understood [57–59]. Most commonly, LHON is caused by point mutations in the NADH dehydrogenase subunit 4 (ND4) gene (G11778A) [60]. ND1 (G3460A), and ND6 (T14484c) mutations are the next most commonly affected. Clinically, patients typically present with sudden, painless central vision loss associated with degeneration of the retinal ganglion cell layer. Previous studies have tested the efficacy of coenzyme Q10 derivatives and vitamin B12 therapy for the treatment of LHON, but with limited and variable success [61–63]. A safety and efficacy gene therapy trial by Wan et al., however, suggested the use of gene therapy as a potentially viable treatment option for LHON [64] (NCT01267422). The study involved nine patients who underwent a single-dose intravitreal injection of rAAV2-ND4, the outcome measures for which were monitored over a 9-month follow-up period. To account for the fact that AAV2 gene delivery is limited to the nucleolus, the authors re-coded the mitochondrial *ND4* gene into nuclear genetic code prior to transducing the vector. Six out of the nine patients experienced significant improvement in BCVA while three of the patients reported improvement in their visual field; all other parameters remained stable. No local or systemic adverse events were observed. While a larger sample size and longer follow-up are needed to further evaluate the potential for this gene therapy as a treatment for LHON, the study provides promising preliminary data suggesting the safety and efficacy of intravitreal rAAV2-ND4 injection.

II.d. Age-related macular dystrophy (AMD) gene therapy results:

Age-related macular dystrophy (AMD) is a common cause of irreversible visual loss in the United States [65]. Exudative AMD is one manifestation of the disease, which is defined by choroidal neovascularization (CNV). Conventional treatment has focused on antagonizing vascular endothelial growth factor (VEGF), a pro-angiogenic signaling molecule, with intravitreal injections of drugs like aflibercept and bevacizumab; however, these injections have limited effect duration and patients typically require regular follow-up for repeat injections. Gene therapy provides an opportunity for a more durable treatment by increasing native expression of VEGF antagonists. A phase I trial tested the delivery of genes for two anti-angiogenic factors—endostatin, a cleavage product of collagen XVIII, and angiostatin, a cleavage product of plasminogen—via a lentivirus injected subretinally [66] (NCT01301443). Results demonstrated a decrease in leakage on fluorescein angiography; however, this was not associated with further anatomical (central subfield thickness) or visual significance. The therapy appeared safe and the study reported no significant adverse effects or inflammatory reactions, although there were several complications related to the subretinal injection, including the formation of one macular hole. Phase I and II trials have also tested gene therapy with sFLT, a soluble version of a VEGF receptor that, in effect, works as a VEGF trap [67–69] (NCT01494805). Delivered subretinally in an AAV, the agent showed trends toward more consistent stabilization and, in some cases, improvement in visual acuity compared to conventional therapies. Additionally, patients randomized to the intervention arm of a 32-patient phase II trial required fewer injections of conventional medications as compared to the control group. This same trial reported three cases of anterior uveitis; however, these were not visually significant and the intervention appeared to be safe otherwise. Intravitreal injection of AAV2-sFLT01 proved to be relatively safe and well-tolerated in another study; notably, six out of 19 patients exhibited reduced subretinal fluid and significant improvement in vision, although two experienced potentially drug-related adverse events [70] (NCT01024998). Larger phase III studies will be needed to assess the efficacy of these gene therapies as compared to conventional treatment.

III. Genome Surgery:

The term genome surgery relates to CRISPR gene editing which has the ability to edit, delete, and epigenetically modify DNA *in vivo* [5, 23, 25]. Although both traditional gene therapy and genome “surgery” require intraoperative sub-retinal delivery, the term “genome surgery” was coined as a depiction of the molecular process taking place. With traditional gene therapy, a wildtype gene is *supplemented*, whereas CRISPR edits the endogenous genetic code to create what is believed to be a permanent change. Here we describe briefly CRISPR mechanism to demonstrate its therapeutic potential for still unapproachable retinal dystrophies. We recommend additional literature for in depth discussion of CRISPR genome surgery [71–73], and in the retina [5, 23, 25, 30].

The modification of pathogenic alleles begins with DNA double-strand breaks (DSBs) created by Cas9 endonuclease at target sequences, directed by the guide RNA (gRNA). Repair then occurs by one of two mechanisms: in homology-directed repair, editing is accomplished by introducing wild-type allele to be used as a template, while, in non-

homologous end joining (NHEJ), “knock out” results because no donor template is used and DSB repair proceeds haphazardly, causing insertions and deletions (INDELs). Gene silencing by NHEJ results from shifts in the reading frame caused by INDELs, often creating premature stop codons. NHEJ may prove useful in cases of dominantly-inherited disease that are not haploinsufficient. Knocking out the mutated allele may allow the phenotypic reversion to become wild-type.

It is important to note that, while the basic CRISPR mechanism is understood, its action is complex and can yield off-target mutagenesis, or unintended cleavages [30, 74–80]. Efforts to increase on-target and decrease off-target cleavage are being made [76, 81–84]: these include improving gRNA design [83, 85–87] and engineering high-fidelity variants of Cas endonuclease [81, 88–93]. Although an imperfect tool, the retina may be the most appropriate setting for CRISPR clinical trials, as it has been for gene therapy; the retina enjoys relative immune- privilege from the blood-retina barrier [20] and can be viewed at the cellular level by imaging such as OCT without invasive techniques [1, 23–25]. Still, it is hoped that more efficient CRISPR genome surgery will be achieved in order for CRISPR/Cas9’s potential to be harnessed to the fullest extent.

IV. Conclusion:

In recent years, advances in gene therapy have brought the technology to phase I, II, and III trials. Thus far, gene therapy appears to be safe, as seen in trials for LCA, CHM, and exudative AMD, and potentially efficacious for a timeframe of months to years, although caution remains due to the less-common potential for inflammatory reactions and surgical complications. The FDA approval of a gene therapy for *RPE65*-associated disease brings increasing anticipation for further clinical applications of gene therapy and, eventually, genome surgery. Larger clinical trials will improve understanding of the efficacy and safety profiles of these medications, which may be vision-saving drugs for previously untreatable IRDs.

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Key Points:

- Gene therapy has potential to provide therapeutic options for previously unapproachable retinal dystrophies.
- Gene therapy clinical trials have varied results but show potential to be therapeutic.
- While gene therapy cannot be applied to all inherited retinal degenerations, genome surgery may be able to meet the need for therapeutic options.

Table 1.

Completed Retinal Degeneration Gene Therapy Trials as of August 2018

NCT ID	Dystrophy	Sub-retinal Injection	Phase	Citations
00643747	LCA2	tgAAG76 (rAAV 2/2.hRPE65p.hRPE65)	I,II	[16, 37]
01496040	LCA2	rAAV2/4.hRPE65	I,II	[46]
00821340	LCA2	rAAV2-hRPE65	I	Not resulted
00999609	LCA2	voretigene neparvovec-rzyl (AAV2-hRPE65v2-101)	III	[47]
00749957	LCA2	rAAV2-CB-hRPE65	I,II	[48]
02077361	CHM	rAAV2.REP1	I,II	[55]
01461213	CHM	rAAV2.REP1	I,II	[33]
01301443	Wet AMD	RetinoStat (lentivirus with endostatin/angiostatin)	I	[66]
01494805	Wet AMD	rAAV.sFlt-1	I	[67]
			I	[68]
			IIa	[69]
01024998	Wet AMD	AAV2-sFLT01	I	[70]
01267422	LHON	rAAV2-ND4	n/a	[64]

Completed trials of gene therapy are listed. Of note, most gene therapies follow a delimited naming convention in which the first part of the name indicates the type of viral vector and, the last, the gene being delivered. Where this is not clear, parenthetical comments have been added. Abbreviations: LCA, Leber congenital amaurosis; CHM, Choroideremia; AMD, age-related macular degeneration; LHON, Leber hereditary optic neuropathy.

Table 2.**Retinal Degeneration Gene Therapy Trials in Progress**

NCT ID	Disease	Injection Location	Intervention	Phase
02946879	LCA2	Subretinal	AAV OPTIRPE65	I,II
00516477	LCA2	Subretinal	voretigene neparvovec-rzyl (AAV2-hRPE65v2-101)	I
01208389	LCA2	Subretinal	voretigene neparvovec-rzyl (AAV2-hRPE65v2-101)	I,II
00481546	LCA2	Subretinal	rAAV2-CBSB-hRPE65	I
02781480	LCA2	Subretinal	AAV RPE65	I,II
03140969	LCA10	Intravitreal	QR-110	I,II
02064569	LHON	Intravitreal	GS010	I,II
03293524	LHON	Intravitreal	GS010 (rAAV2/2-ND4)	III
03153293	LHON	Intravitreal	rAAV2-ND4	II,III
02161380	LHON	Intravitreal	scAAV2-P1ND4v2	I
03428178	LHON	Intravitreal	rAAV2-ND4	n/a
03507686	CHM	Subretinal	AAV2-REP1	II
03496012	CHM	Subretinal	AAV2-REP1	III
03584165	CHM	Subretinal	AAV2-REP1	n/a
02341807	CHM	Subretinal	AAV2-hCHM	I,II
03326336	RP	Intravitreal	GS030-DP (optogenetics with rAAV2.7m8-CAG- ChrimsonR-tdT omato)	I,II
02556736	RP	Intravitreal	RST-001 (optogenetics with channelrhodopsin-2)	I,II
03374657	RP	Subretinal	CPK850 (scAAV8-RLBP1)	I,II
03328130	RP	Subretinal	AAV2/5-hPDE6B	I,II
03252847	X-linked RP	Subretinal	AAV-RPGR	I,II
03116113	X-linked RP	Subretinal	AAV-RPGR	I,II
03316560	X-linked RP	Subretinal	rAAV2tYF-CB-RPGR (ORF15 gene)	I,II
02416622	X-linked RS	Intravitreal	rAAV2tYF-CB-hRS 1	I,II
02317887	X-linked RS MERTK-	Intravitreal	AAV8-sdRS/IRBPhRS	I,II
01482195	associated Retinal Disease	Subretinal	rAAV2-VMD2-hMERTK	I
03066258	Wet AMD	Subretinal	RGX-314 (AAV8 with gene encoding anti-VEGF monoclonal antibody fragment)	I
01024998	Wet AMD	Intravitreal	AAV2-sFLT01	I
03585556	Wet AMD	Intravitreal	AAVCAGsCD59	I
01678872	Wet AMD	Subretinal	RetinoStat (lentivirus with endostatin/angiostatin)	I
03144999	Dry AMD	Intravitreal	AAVCAGsCD59	I
03284268	Retinoblastoma	intravitreal	VCN-01 (oncolytic adenovirus)	I

In progress trials of gene therapy are listed. Of note, most gene therapies follow a delimited naming convention in which the first part of the name indicates the type of viral vector and, the last, the gene being delivered. Where this is not clear, parenthetic comments have been added.

Abbreviations: LCA, Leber congenital amaurosis; LHON, Leber hereditary optic neuropathy; CHM, Choroideremia; RP, retinitis pigmentosa; MERTK, MER tyrosine kinase protooncogene; AMD, age-related macular degeneration.