Purpose of review
Highlight some of the recent advances in gene therapy and gene modification for optic nerve disease to promote axon regeneration, neuroprotection, and increased visual functioning.

Recent findings
Visual loss secondary to optic nerve damage occurs in numerous ophthalmologic and neurologic conditions. Damaged retinal ganglion cells (RGCs) do not regenerate once they undergo apoptosis after injury. Gene therapy has been studied to replace gene mutations in disorders affecting the optic nerve as well as to alter genes responsible for suppressing or activating pathways of optic nerve growth and regeneration. Recent clinical trials for Leber’s Hereditary Optic Neuropathy have demonstrated safety and feasibility as potential future treatment. Animal studies utilizing gene therapy for optic nerve regeneration have shown various degrees of RGC axon regrowth and target reinnervation. Some studies have also successfully demonstrated a state of neuroprotection in RGCs allowing them to survive in greater numbers following injury.

Summary
Additional studies will have to evaluate long-term efficacy and safety of these potential treatments, as well as the consequences of manipulating tumor suppressor genes and oncogenes.

Keywords
axon regeneration, gene therapy, Leber’s hereditary optic neuropathy, neuroprotection, optic nerve, optic neuropathy, retinal ganglion cell

INTRODUCTION
Optic neuropathies are a broad category of disease with many causes. Causes include ischemia, inflammation, toxicity, nutritional deficiencies, glaucoma, compression, trauma, hereditary, congenital disorders, or as part of a larger neurodegenerative process. Although these disease processes have characteristic features, timing, and patterns of visual loss, they all result in the death of retinal ganglion cells (RGCs) [1–3]. RGCs are the cell bodies of the axons that form the optic nerve itself. Unlike neurons within the peripheral nervous system, adult central nervous system (CNS) neurons, including RGCs, do not regenerate after injury [2,3]. Many factors limit RGC axon regeneration and involve both extrinsic and intrinsic barriers. Extrinsic limitations are because of the inhibitory environment for axon regeneration created after RGC axonal injury. Oligodendrocytes, the myelinating cells of the CNS, secrete inhibitory proteins and molecules that impede axon regrowth [1–3,4]. This differs from the peripheral nervous system where the myelinating Schwann cells promote axon regeneration [3]. Astrocytes also limit axon regeneration by releasing inhibitory molecules and by forming glial scars that may act as physical barriers to axon regrowth. The primary intrinsic barrier is that mature RGCs, like other CNS neurons, lack sufficient intrinsic ability to grow and regenerate. Many genes responsible for robust cellular proliferation and axon growth are active in embryonic RGCs; however, these genes become heavily suppressed in mature RGCs [1,3]. Axonal damage also interrupts the transport of neurotrophic factors from target cells, resulting in the increase of proapoptotic proteins in RGCs [1,5–7]. These factors usually make visual recovery from optic nerve disease and injury impossible.

Gene therapy is a rapidly evolving area utilizing viral and nonviral means to transfer genetic material or manipulate gene expression toward a therapeutic goal. This may include transferring normal genes...
Gene therapy clinical trials for LHON have shown safety and feasibility of the AAV2-ND4 viral vector.

Animal studies suggest viral and nonviral gene therapies may be potential options for optic nerve regeneration and neuroprotection in the future.

Sustained efficacy and long-term risks such as tumor formation are issues that will have to be further evaluated.

Gene therapy in optic nerve disease

KEY POINTS

- Gene therapy clinical trials for LHON have shown safety and feasibility of the AAV2-ND4 viral vector.
- Animal studies suggest viral and nonviral gene therapies may be potential options for optic nerve regeneration and neuroprotection in the future.
- Sustained efficacy and long-term risks such as tumor formation are issues that will have to be further evaluated.

LHON is a hereditary optic neuropathy caused by point mutations in mitochondrial DNA. The three most common occur at nucleotide positions 11778, 14484, and 3460. A point mutation at 11778 in NADH dehydrogenase, subunit 4 (ND4) gene is most common, accounting for 60–90% of LHON patients [10–14]. Patients are typically young men with simultaneous or sequential, painless vision loss [10]. Diseases because of single-gene mutations are good candidates for gene therapy, and the 11778 mutation has been the primary focus as it is most common and has the lowest likelihood of spontaneous visual recovery [8,10,12]. Adeno-associated viruses type 2 (AAV2) have been the vectors utilized given their safety and high efficiency of transduction to inner retinal layers, including RGCs, after intravitreal injection [8,9,15,16]. They also have a low risk of tumor formation from insertional mutagenesis [16]. Clinical trials have used intravitreal injections of recombinant AAV2 carrying ND4 (rAAV2-ND4) in hopes that the normal gene expressed within RGCs will restore or rescue function [12,14,17,18,19,20].

Wan et al. [12], enrolled nine patients in a prospective study where the eye with the worse visual acuity was injected with a single dose of rAAV2-ND4. Patients had 12 months of clinical observation without spontaneous recovery prior to treatment. Improvements began after 3 and 6 months of follow-up. At nine months, seven of the nine patients had improved best-corrected visual acuity (BCVA) of at least 0.3 log MAR. Visual field index was improved in seven of the nine injected eyes. One did not improve, and one refused visual field testing. Electoretinogram data was unable to be adequately ascertained. However, visual evoked potential (VEP) P100 amplitudes were increased in seven of the nine injected eyes. Optical coherence tomography (OCT) showed no significant change in retinal nerve fiber layer (RNFL) thickness [12]. Yang et al. [18] monitored these same patients over a 36-month period. BCVA of two injected eyes that initially had improvement worsened after 6 months, but uninjected eyes had improvement that was maintained over the 36-month monitoring period. One patient with bilateral improvement initially returned to baseline after 12 months. Two patients had sustained improvement of 0.2 log MAR in uninjected eyes. Visual field parameters improved in injected and uninjected eyes of four patients but decreased after 6 months. Two patients had sustained improvements in visual field. Although the VEPs had improvements, P100 latency improvements returned to baseline over time, and amplitude increases were not statistically significant. OCT RNFL measurements in injected eyes remained stable from baseline over the 36-month period, but uninjected eyes had a significant decrease from baseline [18]. No significant safety concerns were identified as no ocular or systemic adverse events were encountered and no decrease in vision below baseline was observed. However, the results show that at least some of the potential benefit may be transient, suggesting expression of the transferred gene may diminish with time.

An open-label dose escalation study using AAV2-ND4 scheduled to end in 2019 has published initial data. The primary outcome measure is toxicity. There are three study arms: patients in group 1 have bilateral vision loss to at least 20/200 and 12 or less months from onset in one eye and at least 6 months in the other, patients in group 2 have bilateral vision loss less than 20/200 and less than 12 months from onset in both eyes, and patients in group 3 have vision loss to less than 20/200 for less than 12 months in one eye and vision 20/40 or less in the other eye. In group 3 the better eye is injected. Eyes must have at least light perception vision. Each group is further stratified into low, medium, and high-dose vector categories [19,20].

Feuer et al. [19], published results of the first five patients followed for 90–180 days. Four patients fell into group 1, three of whom received low dose of vector and one received medium dose in one eye.
The fifth patient fell into group 2 and received low dose. The three patients from group 1 who received low dose of vector did not have a change in vision. However, the group 1 patient who received medium dose and the group 2 patient who received low dose both improved by three lines of BCVA by 3 months. No patients lost vision, and adverse events were minor, including increased intraocular pressure, toxic keratitis, subconjunctival hemorrhage, and sore throat [19].

Guy et al. [20], published results for low and medium-dose vector on 14 patients followed up to 18 months, including the five from the initial publication. A change of 0.1 log MAR was equal to one line of vision or five letters on Early Treatment Diabetic Retinopathy study. Over 18 months for group 1 and 2 patients, treated eye changes ranged from 0.19 to 0.45 log MAR, whereas untreated eye changes ranged from 0.08 to 0.13 log MAR. One of six patients improved in group 1 and three of six improved in group 2. Group 2 treated eyes had a higher rate of improvement than untreated worse eyes in their natural history study [20,21]. There were two group 3 patients, one of which lost vision. By spectral-domain OCT, average temporal RNFL measurements increased one micron at 12 months from baseline, whereas untreated eyes decreased an average of six microns [20].

RESCUE and REVERSE using rAAV2/2-ND4 intravitreal injection for 11778 mutation are in Phase III. RESCUE for vision loss less than 6 months and REVERSE for more than 6 months to 1 year. Patients must be at least 15 years of age and have at least count fingers vision. The study is randomized and double masked. Patients are given intravitreal injection of vector vs. sham injection. Initial data from this study is pending [22].

**GENE THERAPY IN OPTIC NERVE REGENERATION AND NEUROPROTECTION**

As discussed, the RGCs cannot regenerate once injury has occurred. Numerous genes that promote and inhibit RGC regeneration and survival have been recently described and there have been many animal studies evaluating the effects of altering them.

The mammalian target for rapamycin (mTOR) is thought to be a powerful intrinsic regulator of CNS axonal growth and regeneration. It appears to be heavily inhibited in mature CNS neurons, in part by a negative regulator called phosphatase and tensin homolog (PTEN) as well by suppression of cytokine signaling 3 [4,16,23,24]. The mTOR pathway plays a large role in stem cell proliferation in the embryonic CNS. The PTEN/mTOR pathway has, therefore, been the focus of several murine studies for optic nerve regrowth. PTEN deletion in mice has shown increased RGC survival and axon regeneration following optic nerve crush (ONC) injury [25]. One study used short hairpin RNA delivered via AAV2 vector to silence PTEN in RGCs. It showed increased survival of RGCs and long-distance axon regeneration as far as the chiasm following ONC [26]. Some earlier studies have demonstrated significant axon regeneration with PTEN/suppression of cytokine signaling 3 deletion, an effect enhanced in conjunction with cyclic adenosine monophosphate-analogue and Zymosan injection [23,24,27]. Zymosan increases oncomodulin secretion by inflammatory cells, stimulating axon regeneration [4,27,28]. Another study demonstrated impressive results utilizing ras homolog enriched in brain 1, a positive regulator of mTOR, in conjunction with high-contrast visual stimulation. Mice received intravitreal injection with AAV-ras homolog enriched in brain 1 two weeks prior to ONC. Mice subjected to monocular visual stimulation of the injured eye demonstrated RGC axon regeneration beyond the chiasm to target connections in the lateral geniculate nucleus and pretectal nuclei with partial recovery of visual behavior [29].

The Rho/Rho-associated coiled-coil-containing protein kinase (ROCK)/LIMK pathway is also a powerful mediator of CNS neuron growth and regeneration. ROCK is an important intracellular mediator promoting cell death and axon degeneration. ROCK is activated by ras homolog family member A (RhoA), which in turn is activated by several extracellular growth inhibitors [16,30]. Animal studies have shown increased RhoA/ROCK expression in traumatic and vascular optic nerve injury as well as increased levels in RGCs of glaucoma patients [31,32]. RhoA has become a target of interest for downregulation as it lowers intracellular ROCK after axonal injury. Fasudil, a ROCK inhibitor, has been shown to reduce RGC apoptosis after axonal injury in animal models. One study used rabbits with ONC and evaluated the effects of fasudil on RGCs in vitro and in vivo. Control rabbits showed increased RhoA/ROCK expression in injured eyes suggesting the proapoptotic pathway is activated by axonal injury. Reduced RhoA and ROCK expression was associated with increased survival of RGCs in vitro and in vivo [33]. Another study specifically downregulated RhoA expression in rats with ONC using short hairpin RNA via AAV vector injected intravitreally. RGC survival and axon regeneration were significantly increased compared with controls [31]. One study evaluated a topical ROCK inhibitor with intraocular pressure lowering effects on rats after ONC. RGCs of rats that received drug had significantly increased
survival and axon regeneration. The effect on target gene expression was also assessed by measuring phosphorylation of target proteins using western blot. Two downstream targets, coflin and LIM kinase (LIMK), were reduced compared with placebo [34*]. This and other studies suggest the possible utility for topical ROCK inhibitors in the treatment of glaucoma.

Neuroglobin (Ngb), a type of heme protein with high affinity for oxygen, is thought to act mostly as a neuroprotectant within the CNS by binding oxygen-free radicals [35–39]. Ngb concentrations are around 100 times higher in RGCs than other areas of the CNS, likely because of high mitochondrial energy output within the unmyelinated RGC axons [36,37]. However, levels acutely reduce following optic nerve injury [37]. Mitochondrial enzymatic activity has shown to be decreased in mice with glaucoma even before neuronal loss. Intravitreal injection of AAV2/2-NGB in 2-month old mice with glaucoma prevented neuronal loss and maintained mitochondrial function, whereas injection in 8-month old mice showed increased mitochondrial function in remaining RGCs but neuronal loss to that point was not prevented [40]. Another study injected chimeric Ngb into mouse eyes before ONC; it showed three times more RGC survival compared with controls and even enhanced axonal regeneration beyond the crush site [36].

The Kruppel-like factor family are a group of transcription factors that inhibit axon growth [41,42]. They can prevent neurite outgrowth independent of RGC survival [42,43*]. In one recent study, AAV2 expressing anti-Kruppel-like factor9 was injected into mouse eyes before optic nerve injury. This was followed by injection of TPEN after ONC, and fibers grew half the length of the optic nerve [43*]. Administration of TPEN, a Zn$^{2+}$ chelator, has been shown to increase RGC survival and axon regeneration after optic nerve injury. Zinc concentrations increase in the interneurons and eventually RGCs after optic nerve injury and have been implicated to play a key role in cell death [43*,44]. Compared with controls and individual treatments, this combination had the most robust axon regenerative results. Some fibers grew half the length of the optic nerve at two weeks postinjury, and fibers grew to the chiasm and a small amount into ipsilateral optic tract at 6 weeks [43*]. One of the most significant points of interest of this study is achieving these results without altering oncogenes or tumor suppressors.

REGROWTH TO TARGETS AND NEWER TECHNOLOGIES

One of the most challenging limitations to regenerating RGC axons by any method, including targeted gene therapies, is accurately navigating and pairing with target connections in the brain. Although many animal studies demonstrate impressive amounts of axon regeneration, they also show evidence of misdirected regrowth. Many axons take circuitous paths and even reverse direction within the optic nerve. They have been shown to take inconsistent paths within the chiasm and even grow down the opposite optic nerve [27,43*,45,46]. It is unclear at this point whether these misdirected fibers could synapse with inappropriate targets and result in erroneous visual function.

Less toxic but currently less efficacious nonviral gene delivery methods are beginning to show promise, as well as newer technologies such as Clustered Regularly Interspaced Short Palindromic Repeats [47,48,49]. These technologies have not yet been utilized for optic nerve disorders.

CONCLUSION

Targeted gene therapy for optic nerve disease has made promising advances in experimental animal models and early human trials. There are, however, challenges beyond genetically enabling RGC axons to regenerate that remain. Gene expression must persist long enough for adequate therapeutic effect and subsequently not succumb to regression. Similarly, long-term expression would be required in the case of genetic disease. Regenerating RGC axons must be stimulated and guided to take the correct course through the afferent visual pathway and synapse with the correct target neurons in the brain. Risk of tumor formation with long-term manipulations of some of these oncogenes and tumor suppressor genes will have to be addressed.

Acknowledgements

None.

Financial support and sponsorship

Dr. Moster has received research support from Gensight Biologics.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Review on optic nerve regeneration that covers some related topics including genetically regulated regenerative pathways.


9. This is an excellent overview of current gene therapy in multiple eye diseases.


18. The review provides a very complete overview of the numerous pathways involved in CNS axon regeneration and studies that have demonstrated success.


24. The review provides a very complete overview of the numerous pathways involved in CNS axon regeneration and studies that have demonstrated success.


27. The study showed long-term safety of AAV vector for LHON over 3 years and promising results suggesting good feasibility of treatment.


30. The study shows safety with low and medium-dose AAV2-N4 vector for LHON and promising visual outcomes.


The study showed a significantly increased transduction efficiency to RGCs with a mutant AAV2 vector versus wild type for optic nerve regeneration.