Review

The Present and Future of Mitochondrial-Based Therapeutics for Eye Disease

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Mitochondrial dysfunction within the eye contributes to primarily mitochondrial diseases affecting the visual system such as Leber hereditary optic neuropathy (LHON) as well as more common ocular diseases, including glaucoma, diabetic retinopathy, retinopathy of prematurity, and age-related macular degeneration (AMD). For these reasons, druggable targets and gene therapies for improving mitochondrial function have been of significant interest within scientific and pharmaceutical endeavors seeking to improve visual outcomes in ocular disease. These therapies modulate mitochondrial functions, including mitochondrial membrane potential and membrane stability, redox signaling and oxidative stress, mitochondrial quality control including fusion/fission and biogenesis/mitophagy, apoptosis, and mitochondrial genetic-based therapies. As of now, several mitochondrial-targeted therapies have been approved in a limited number of countries, including photobiomodulation for AMD, idebenone for LHON, and SkQ1 for dry eye disease. Elamipretide for nonexudative AMD and gene therapy with GS010 for LHON have additionally shown encouraging results within clinical trials.

Translational Relevance: Mitochondria are viable therapeutic targets for a broad spectrum of ocular diseases.

Introduction

Inherited mitochondrial diseases are incurable and among the most common inherited neurologic disorders, with a prevalence of ~1 in 4300, and ~1 in 200 carry a known deleterious mitochondrial DNA mutation.[1] The neural retina and retinal pigment epithelium (RPE) are among the most metabolically active tissues in the body[2] and are commonly affected in primarily mitochondrial diseases affecting the visual system, including Leber hereditary optic neuropathy (LHON), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), chronic progressive external ophthalmoplegia, and others. Mitochondrial dysfunction within the neural retina and RPE also contributes to more common ocular diseases, including glaucoma, diabetic retinopathy (DR), retinopathy of prematurity, and age-related macular degeneration (AMD), the leading cause of vision loss in the elderly, affecting 150 million people globally.[3] For these reasons, druggable targets and gene therapies for improving mitochondrial function have been of significant interest within scientific and pharmaceutical endeavors seeking to improve visual outcomes in ocular disease.

Mitochondria are double-membrane-bound organelles present in all eukaryotic cells that function primarily in cellular energy metabolism. This is largely driven by a complex interplay between mitochondria and the entire cell, involving proteins encoded by both mitochondrial DNA (mtDNA) and nuclear encoded mitochondrial targeted proteins.[4] As electrons pass through the inner-membrane-bound electron transport

chain (ETC) and ultimately to oxygen, a proton gradient is established that is used to generate the majority of cellular adenosine triphosphate (ATP). Mitochondria also function in intracellular calcium signaling, orchestrate redox reactions and reactive oxygen species (ROS) signaling, initiate apoptotic cell death, and contribute to the synthesis of iron-sulfur clusters.[5] Adding to these intrinsic mitochondrial activities, processes that alter mitochondria size from big (through fusion with adjacent mitochondria) to small (through fission or splitting of mitochondria membranes) and the number of mitochondria per cell (through opposing processes called biogenesis and mitophagy) also influence cellular health and disease.

This review will focus on mitochondrial-targeted therapies in pre/clinical settings being explored for ocular disease. Stem cell therapies have also been applied to ophthalmic mitochondrial disease in the context of cellular replacement/regeneration[6] and via mesenchymal stem cell exosomes providing paracrine/trophic support and mitochondrial transfer,[7] but a comprehensive analysis of stem cells is outside of the scope of this review.[8,9] While there is considerable overlap between mitochondrial physiologic pathways, we have grouped mitochondrial-targeted ocular therapies by their most relevant mechanistic pathway. The five pathways that we review are (1) mitochondrial membrane potential and membrane stability (Fig. 1A), (2) redox signaling and oxidative stress (Fig. 1B), (3) mitochondrial quality control including fusion/fission and biogenesis/mitophagy (Fig. 1C), (4) apoptosis (Fig. 1D), and (5) mitochondrial genetic-based therapies (Fig. 1E).

Mitochondrial Membrane Potential and Membrane Stability

Oxidative phosphorylation is the most efficient method of ATP production in mammalian cells[10] and one of the most well-defined and understood functions of mitochondria. Briefly, this process takes place along the inner mitochondrial membrane via a set of ordered biochemical reactions between numerically named protein complexes (Fig. 1A). These ordered reactions are driven in part by a shuttling of electrons between the complexes, coupled with the pumping of hydrogen protons into the intermembrane space. This generates a proton gradient and a positive membrane potential in the mitochondrial intermembrane space used by complex V, also known as ATP synthase, to drive the production of ATP.[11] Given that ATP is one of the most critical molecules for sustaining cellular functions

and general viability, it is not surprising that diseases that disrupt mitochondrial membrane potential by altering complex function or membrane stability are well-established sources of visual system degenerative events. Therapeutic molecules targeting the restoration of membrane potential and membrane stability are important interventions to improve visual outcomes in patients with mitochondrial-related disease.

Quinone analogs, such as coenzyme Q_{10} (CoQ10), and the more efficacious, shorter chain quinones with better bioavailability and pharmacokinetics such as idebenone and EPI-743 are being explored as therapies targeting the electron chain transport and promoting ATP synthesis. These compounds are being explored in a variety of ocular diseases, including AMD, DR, diabetic macular edema (DME), Fuchs corneal dystrophy, and LHON. LHON is a prototypical primary mitochondrial disease caused by mutations in mtDNA and is the most common primary mitochondrial disease. Missense mutations of genes encoding for complex I are responsible for 90% to 95% of LHON cases,[12] usually a G to A point mutation at nucleotide 11778 in mtDNA that results in a dysfunctional ND4 subunit. Each mutation causing LHON affects one of the subunits of complex I of the ETC, resulting in dysfunctional oxidative phosphorylation and impaired ATP production. In contrast to other mitochondrial diseases that determine a multisystemic phenotype, LHON often manifests as a nonsyndromic purely ocular disease. Unlike most primary mitochondrial diseases, LHON manifests with all mtDNA being involved to the same degree (i.e., homoplasmic).[13] These mutations cause subacute loss of retinal ganglion cells (RGCs), leading to central vision loss and, in some cases, blindness.

The mechanism of action of quinone analogs is through bypassing mitochondrial complex I inhibition and shuttling electrons from the cytosol directly to complex III, thereby reestablishing ATP synthesis and ultimately reducing cytochrome *c*.[14] The Rescue of Hereditary Optic Disease Outpatient Study (RHODOS; NCT00747487), a multicenter, double-blind, randomized, placebo-controlled trial, enrolled patients with LHON with onset within 5 years to receive oral idebenone. This study failed to show a significant difference in visual acuity (VA) between the groups at 24 weeks. However, a subgroup analysis revealed significantly better functional results in patients with discordant VA (i.e., early disease). Only a single serious adverse event consisting of infected epidermal cyst in the idebenone group occurred in the cohort of 55 patients treated with idebenone.[15] Furthermore, the RHODOS Follow-up Single-visit Study (RHODOS-OFU; NCT01421381) showed persistent beneficial effects with stable VA
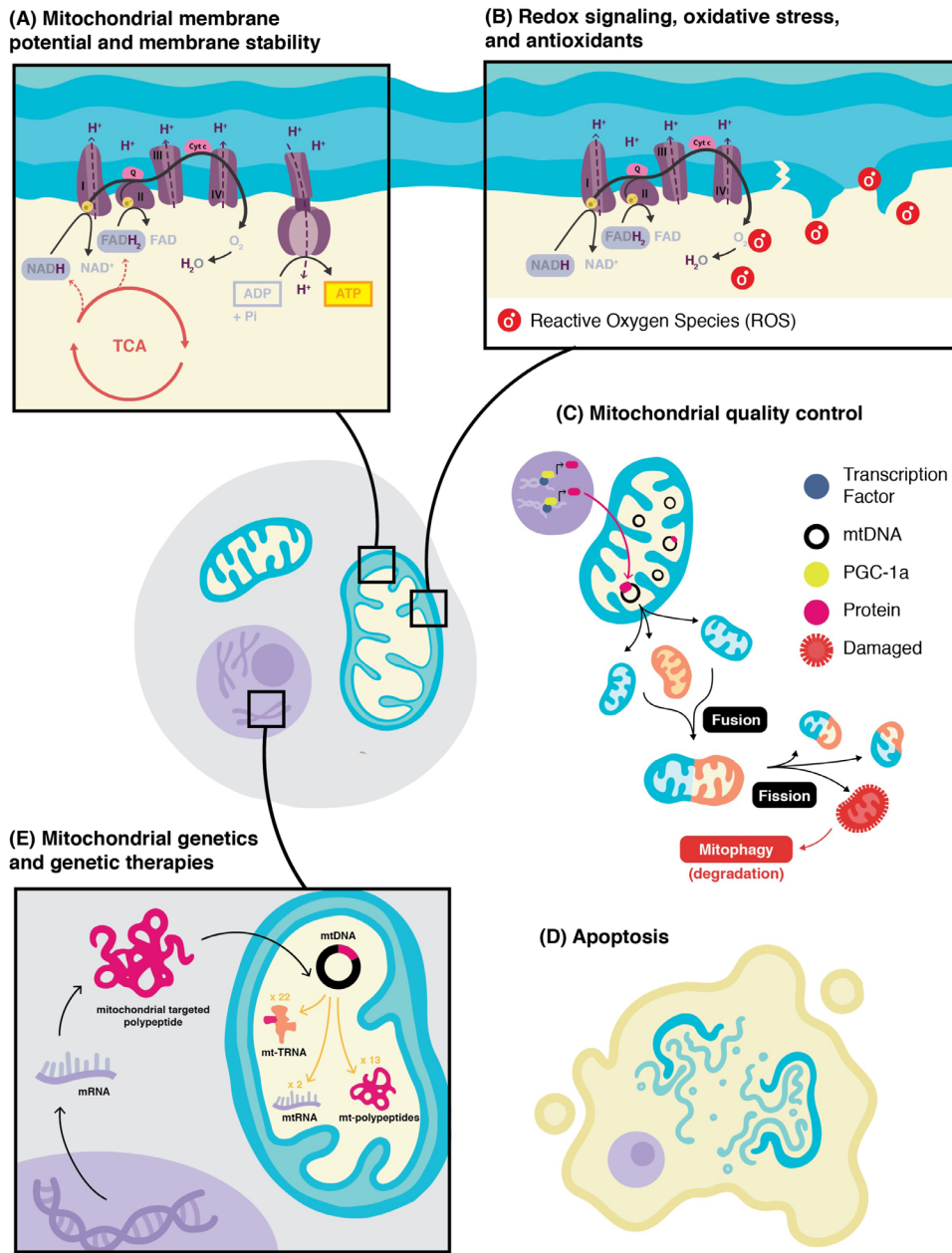
Figure 1. Mitochondrial functions and associated activities critical to cellular homeostasis/survival, subdivided by discussed theraputic targets and intervention.

or with some improvements after discontinuation of idebenone for 30 months.[16] This is possibly due to the fact that early treatment protects RGCs during the acute phase of disease when the most damage occurs, and thus RGCs tend to remain more robust on a longer term basis compared to controls. A large expanded access program from November 2011 to September 2015 showed that the drug provides objectively functional improvements as early as 6 months, and although there was no control group, the proportion of patients with visual recovery was higher than what reported in the literature.[17]

Based on the clinical trials above, idebenone (Raxone, Santhera Pharmaceuticals (Pratteln, Switzerland)) is the first and only approved treatment for LHON but limited to the European Union, Norway, Iceland, Liechtenstein, Israel, and Serbia (Table 1). Currently, long-term efficacy and safety for patients with onset within 1 year are subjects of the phase IV Study to Assess the Efficacy and Safety of Raxone in LHON Patients (LEROS; NCT02774005), and visual outcomes will be compared to those of an external natural history control group. Other optic neuropathies might share mitochondrial dysfunction in their

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Table 1. Clinical Studies Targeting Mitochondria in Ophthalmology

Intervention	Name	Condition Treated	Trial Phase	Trial Code	Status	Reference
Ibedenone (Raxone)	Rescue of Hereditary Optic Disease Outpatient Study (RHODOS)	LHON	II	NCT00747487	Completed	15
Ibedenone (Raxone)	RHODOS Follow-up Single-Visit Study (RHODOS-OFU)	LHON	NA	NCT01421381	Completed	16
Ibedenone (Raxone)	Study to Assess the Efficacy and Safety of Raxone in LHON Patients (LEROS)	LHON	IV	NCT02774005	Ongoing	NA
SkQ1 (Visomitin)	A Clinical Study to Assess the Safety and Efficacy of an Ophthalmic Solution (SkQ1) in the Treatment of Dry Eye Syndrome	Dry eye syndrome	II	NCT02121301	Completed	NA
SkQ1 (Visomitin)	Study of SkQ1 as Treatment for Dry-Eye Syndrome (VISTA-1)	Dry eye syndrome	III	NCT03764735	Completed	NA
SkQ1 (Visomitin)	Vehicle-Controlled Study of SkQ1 as Treatment for Dry-Eye Syndrome (VISTA-2)	Dry eye syndrome	III	NCT04206020	Ongoing	NA
Elamipretide (MTP-131, SS-31, Bendavia)	A Study of MTP-131 Topical Ophthalmic Solution in Subjects with Diabetic Macular Edema and Non-Exudative Intermediate AMD	DME and AMD	I/II	NCT02314299	Completed	NA
Elamipretide (MTP-131, SS-31, Bendavia)	An Open-Label, Phase 1 Clinical Study to Evaluate the Safety and Tolerability of Subcutaneous Elamipretide in Subjects with Intermediate AMD (ReCLAIM)	AMD	I	NCT02848313	Completed	34
Elamipretide (MTP-131, SS-31, Bendavia)	Study to Evaluate Safety, Efficacy & Pharmacokinetics of Elamipretide in Subjects with AMD With Non-central GA (ReCLAIM-2)	AMD	II	NCT03891875	Ongoing	NA
Elamipretide (MTP-131, SS-31, Bendavia)	A Study Investigating the Safety, Tolerability, and Efficacy of Elamipretide Topical Ophthalmic Solution for the Treatment of Fuchs' Corneal Endothelial Dystrophy (ReVEAL)	FCED	I/II	NCT02653391	Completed	NA
Elamipretide (MTP-131, SS-31, Bendavia)	A Study Investigating the Safety, Tolerability, and Efficacy of Elamipretide (MTP-131) Topical Ophthalmic Solution for the Treatment of C12 (ReSIGHT)	LHON	II	NCT02693119	Completed	35
PBM	Near-Infrared Light-Emitting Diode (NIR-LED) Therapy for LHON	LHON	I/II	NCT01389817	Terminated	NA
PBM	Toronto and Oak Ridge PBM Study for Dry AMD (TORPA I)	AMD	II	NCT00940407	Completed	45
PBM	Toronto and Oak Ridge PBM Study for Dry AMD (TORPA II)	AMD	NA	NA	Completed	46
PBM	Study of Photobiomodulation to Treat Dry AMD (LIGHTSITE I)	AMD	NA	NCT02725762	Completed	47
PBM	Study of Photobiomodulation to Treat Dry AMD (LIGHTSITE II)	AMD	NA	NCT03878420	Ongoing	NA
PBM	Study of Photobiomodulation to Treat Dry AMD (LIGHTSITE III)	AMD	NA	NCT04065490	Ongoing	NA
PBM	Study of Photobiomodulation Effect on Electroretinogram Outcomes in Dry AMD (ELECTROLIGHT)	AMD	NA	NCT04522999	Ongoing	NA
GS010 (lenadogene nolparvovec, Lumevoq)	Safety Evaluation of Gene Therapy in LHON Patients	LHON	I/II	NCT02064569	Completed	134
GS010 (lenadogene nolparvovec, Lumevoq)	Efficacy Study of GS010 for the Treatment of Vision Loss up to 6 Months from Onset in LHON Due to the ND4 Mutation (RESCUE)	LHON	III	NCT02652767	Completed	135
GS010 (lenadogene nolparvovec, Lumevoq)	Efficacy Study of GS010 for Treatment of Vision Loss from 7 Months to 1 Year from Onset in LHON Due to the ND4 Mutation (REVERSE)	LHON	III	NCT02652780	Completed	136
GS010 (lenadogene nolparvovec, Lumevoq)	Observational Registry Study of LHON Affected Patients (REALITY)	LHON	Observational	NCT03295071	Completed	153
GS010 (lenadogene nolparvovec, Lumevoq)	Efficacy & Safety Study of Bilateral IVT Injection of GS010 in LHON Subjects Due to the ND4 Mutation for up to 1 Year (REFLECT)	LHON	III	NCT03293524	Ongoing	NA

NA, Not available.

pathogenesis. Small studies showed that idebenone might be beneficial to other conditions as well. Autosomal dominant optic atrophy (ADOA) is a rare genetic disease characterized by loss of RGCs. Unlike LHON, mitochondrial dysfunction is secondary to mutations of two nuclear genes, *OPA1* and *OPA2*, that encode for mitochondrial proteins embedded in the inner mitochondrial membrane and are involved in mitochondrial fusion, metabolism, apoptosis, and calcium homeostasis.[18] In a small study, all seven patients with ADOA treated with idebenone showed improvement in visual acuity.[19] One patient with Wolfram syndrome with progressive visual loss for at least 5 years before also exhibited visual improvement with idebenone.[20]

EPI-743 (vatiquinone; Vincerinone, Edison Pharmaceuticals (Mountain View, CA)) is a newer ubiquinone analogue structurally similar to idebenone but 1- to 10,000-fold more potent[21] and designed to avoid inhibition of complex I documented with idebenone.[22] In a small open-label trial of patients with LHON, progressive vision loss was arrested and reversed in four of five patients.[23] In another open-label trial, EPI-743 was administered to three patients where one eye was clinically involved. Vision improved in the first eye of all three patients, and in one patient, vision loss did not occur in the fellow eye.[24] Another pilot study in a small Brazilian cohort of six patients with LHON found that EPI-743 improved VA, which remained stable for up to 52 months with a slight decline after discontinuation of the drug.[25]

SkQ1 (Visomitin, Mitotech SKQ (Luxembourg, Luxembourg)) is a short-chain quinone that accumulates in mitochondria and has potent antioxidant effects that protect cardiolipin from oxidation. Cardiolipin is a specific phospholipid expressed only on the inner mitochondrial membrane (IMM) that plays a pivotal role in modulating cristae formation as well as organizing ETC complexes into "supercomplexes" necessary for an optimal electron transfer.[26] Its oxidation leads to loss of cristae with a profound disruption of the ETC and is a trigger for apoptosis.[27] In vitro studies showed inhibition of lipid and protein peroxidation and H_2O_2-induced apoptosis.[28] In animal models, it confers resistance to the development of chronic oxidative stress-induced retinopathy and cataracts and may restore some level of vision in blind animals.[29,30] SkQ1 was approved in Russia in 2012 (Table 1) for dry eye disease after good safety and efficacy results within randomized clinical trials. In the United States, a phase II trial, A Clinical Study to Assess the Safety and Efficacy of an Ophthalmic Solution (SkQ1) in the Treatment of Dry Eye Syndrome (NCT02121301), showed that

Table 2. Mitochondrial-Based Therapies Currently Approved Internationally

Intervention	Indication	Country Approved
PBM	Nonexudative AMD	European Union
Idebenone	LHON	European Union, Norway, Iceland, Liechtenstein, Israel, and Serbia
SkQ1	Dry eye disease	Russia

No therapies are currently approved by the FDA in the United States.

SkQ1 is safe and effective in treating dry eye disease, documented with improved corneal fluorescein staining, lissamine green staining, and reduced symptoms related to dry eye disease.[31] The preliminary results from the phase III Study of SkQ1 as Treatment for Dry-Eye Syndrome (VISTA-1), showing an excellent safety profile along with positive results in controlling conjunctival fluorescein staining and dry eye symptoms, were used to design another phase III trial, Vehicle-Controlled Study of SkQ1 as Treatment for Dry-Eye Syndrome (VISTA-2; NCT04206020), that is currently ongoing. Furthermore, two phase III trials for uveitis and dry AMD are in the pipeline.

Similarly, another potent antioxidant, elamipretide (MTP-131, SS-31; Bendavia, Stealth BioTherapeutics Corp (Newton, MA)), selectively accumulates within the inner mitochondrial membrane and stabilizes cardiolipin, preventing it from oxidative stress and peroxidation. In contrast to Skq1, elamipretide uptake is not mitochondrial membrane potential dependent. In vivo studies in rodent diabetic models showed that elamipretide provided resistance to structural changes and visual decline induced by high glucose and also restored vision in late phases of the disease.[32] In mouse AMD models, elamipretide has shown to reverse vision loss and improve clearance of sub-RPE deposits in ApoE4 mice.[33] The phase I/II trial, A Study of MTP-131 Topical Ophthalmic Solution in Subjects with DME and Non-Exudative Intermediate AMD (NCT02314299), evaluated a topical ophthalmic solution of elamipretide for diabetic macular edema and dry AMD in 21 patients, and additional clinical studies are currently under way due to its potential efficacy in the treatment of DME, nonexudative AMD, Fuchs corneal dystrophy, and LHON (Table 2).

The Open-Label, Phase 1 Clinical Study to Evaluate the Safety and Tolerability of Subcutaneous Elamipretide in Subjects with Intermediate AMD (ReCLAIM;

NCT02848313) evaluated daily subcutaneous abdominal injection of elamipretide in patients with intermediate AMD intended as high-risk drusen (two or more large drusen) or noncentral geographic atrophy (GA) (NCT02848313). No serious adverse events were reported, and the study showed encouraging functional visual results with improved best-corrected visual acuity (BCVA) and low luminance visual acuity at 24 weeks. GA growth rate was slower compared to the natural history reported in the literature.[34] Study to Evaluate Safety, Efficacy & Pharmacokinetics of Elamipretide in Subjects with AMD with Non-central GA (ReCLAIM-2; NCT03891875) is an in-progress phase II double-blind placebo-controlled clinical trial that evaluates daily subcutaneous injection of elamipretide in patients with dry AMD with GA for 48 weeks.

A phase I/II double-blinded trial, A Study Investigating the Safety, Tolerability, and Efficacy of Elamipretide Topical Ophthalmic Solution for the Treatment of Fuchs' Corneal Endothelial Dystrophy (ReVEAL; NCT02653391), studied the safety and tolerability of topical elamipretide for the treatment of Fuchs endothelial dystrophy in patients with mild to moderate corneal edema. The study was divided into two parts, one evaluating 1% elamipretide and the other 3% elamipretide, and the results have not yet been released. Furthermore, A Study Investigating the Safety, Tolerability, and Efficacy of Elamipretide (MTP-131) Topical Ophthalmic Solution for the Treatment of LHON (ReSIGHT), another phase II trial, evaluated 1% elamipretide topical solution twice a day in 12 patients with LHON. No adverse events were recorded, and efficacy data appeared to be preliminarily promising, with final results undergoing further evaluation.[35]

Light therapy has also been shown to stabilize the mitochondrial membrane. The application of low-energy photon irradiation to accelerate wound and soft tissue injury healing has been explored for several decades.[36] The therapeutic application of infrared and near-infrared spectrum radiation, termed *photobiomodulation* (PBM), targets mitochondria and enhances their function in order to attenuate degenerating diseases. The therapeutic benefit from mitochondrial enhancement has been hypothesized to result from intracellular signaling pathways triggered when infrared and near-infrared photons are absorbed by the mitochondrial cytochrome *c* oxidase, resulting in improved mitochondrial energy metabolism, increased cytoprotective factor production, and cell survival.[37] Investigations in rodent models with a variety of eye diseases have demonstrated that PBM attenuates photoreceptor cell death, protects retinal function,

and exerts anti-inflammatory actions by augmenting mitochondrial function and stimulating antioxidant protective pathways.[38]

Various studies detailed below have explored the application of light therapy with PBM in the treatment of AMD, retinitis pigmentosa, retinopathy of prematurity, methanol intoxication, and light-induced retinal damage in rodent models and found improved mitochondrial function and retinoprotection with PBM. A decline in mitochondrial function is common in the aged retina and AMD.[2] After 670 nm PBM, aged and AMD mice models experienced increased mitochondrial membrane potential, increased ATP production, reduced inflammation, and reduced oxidative stress.[39,40] This supports the early stage clinical trials of PBM in patients with AMD. PBM therapy in a rodent model of retinitis pigmentosa increased retinal mitochondrial cytochrome *c* oxidase activity, upregulated the retina's production of a key mitochondrial antioxidant enzyme (manganese superoxide reductase), attenuated photoreceptor cell loss, and improved photoreceptor function.[41] Following intoxication of rodents with methanol, PBM significantly attenuated the retinotoxic effects of methanol-derived formate.[38] Studies have shown that PBM is protective against light-induced retinal degeneration, as measured by electroretinography responses, morphology, and reduction in photoreceptor cell death and inflammation.[3,42,43]

A phase I/II trial, Near-Infrared Light-Emitting Diode (NIR-LED) Therapy for LHON (NCT01389817), attempted to evaluate the use PBM in patients with LHON. However, the study was terminated because none of the four patients were able to fixate. A case series showed that PBM has encouraging functional and structural beneficial effects on both dry and wet AMD.[44] The Toronto and Oak Ridge PBM Study for Dry AMD (TORPA I; NCT00940407) study showed that PBM delivered by the Valeda Light Delivery System (LumiThera, Inc, Poulsbo, WA, USA) was able to provide immediate functional improvements in terms of BCVA and contrast sensitivity.[45] In addition, TORPA II proved that the benefits provided by PBM are documentable anatomically on spectral domain optical coherence tomography with reduction in drusen volume without the development of GA. Patients with better baseline BCVA benefited the most from PBM treatment. In either study, no significant adverse event was reported.[45,46]

These findings were subsequently confirmed by the Study of Photobiomodulation to Treat Dry AMD (LIGHTSITE I; NCT02725762) that showed significant improvement in fixation stability using microperimetry and quality of life with PBM

administered via the Valeda Light Delivery System. Current data suggest that the benefits can be maintained by repeating PBM every 4 to 6 months. On the other hand, anatomic outcomes are delayed, supporting the hypothesis that PBM acts at the cellular level improving the overall cellular function. None of the adverse events occurred in the study was related to the treatment according to the investigator.[47] The Valeda Light Delivery System has been approved for dry AMD by the European Medicines Agency in the European Union but not by the Food and Drug Administration (FDA) in the United States. There are three ongoing clinical trials investigating the use of PBM in AMD in the United States, LIGHTSITE II (NCT03878420), LIGHTSITE III (NCT04065490), and Study of Photobiomodulation Effect on Electroretinogram Outcomes in Dry AMD (ELECTROLIGHT; NCT04522999).

In addition, reports showed that PBM can be an effective treatment for other ocular conditions, including retinitis pigmentosa (RP) and even amblyopia. In a patient with RP with baseline BCVA of 20/50, PBM was able to restore vision back to 20/20 as well as nearly completely normalize visual field deficits. Five years after discontinuation, the disease relapsed, but retreatment with PBM yielded similarly improved visual outcomes.[48] In a cohort of adolescents and adults with amblyopia treated with PBM, 90% showed improved BCVA. Even in this case, the magnitude of gain in BCVA was correlated with the baseline BCVA as patients with milder amblyopia responded better to the treatment.[49] Furthermore, in vitro and in vivo studies showed promising results for treating diabetic retinopathy.[50]

Redox Signaling, Oxidative Stress, and Antioxidants

In the process of oxidative phosphorylation, the electron shuttling that drives the proton motive force to generate ATP is terminated by the acceptance of four electrons by molecular oxygen (O_2), splitting O_2 to ultimately generate two water molecules. However, this process is imperfect and occasionally results in premature electron leakage during electron shuttling at complexes I and III.[51] These leaked electrons react with O_2 to form oxygen radicals such as superoxide anions ($\times O_2^-$) and hydroxyl radicals ($\times OH$),[52] collectively referred to as ROS (Fig. 1B). Since these ROS molecules are partially reduced molecules and therefore highly unstable, they can stabilize themselves by oxidizing macromolecules such as proteins, lipids, and

nucleic acids. These oxidation events can often impact the function and stability in proteins and lipids and even induce nucleic acid damage or DNA mutations.[53] As a result, cells have developed mechanisms to reduce the burden of naturally occurring ROS molecules. There are enzyme systems that destroy ROS, such as superoxide dismutase, catalase, and glutathione peroxidase, and there are molecules that act as natural sinks for ROS, such as carotenoids.[53] There is also a set of limited repair systems that reverse the damage to the macromolecules, such as DNA repair mechanisms or protein reductases that remove oxidized groups on proteins.[54,55] Ultimately, all these systems work to reduce the burden of naturally occurring ROS. However, in some diseases, damage to mitochondria or mitochondrial signaling pathways can trigger increased ROS production and overwhelm the capacity of ROS reducing systems, leading to significant degeneration in the visual system.[56] To overcome this problem, targeting therapeutics to improve ROS reducing systems or reduce the production of ROS could significantly improve the retina and associated visual system components.

Many of the therapeutics discussed above function as both mitochondrial membrane stabilizers and antioxidants (coenzyme Q10, idebenone, EPI-743, SkQ1, and elamipretide). Antioxidants have been classically prescribed to reduce the progression of dry AMD to either advanced form of AMD (GA or choroidal neovascular membrane). Reducing oxidative stress with antioxidant supplementation in the Age-Related Eye Disease Study (AREDS)[57] and AREDS2[58] vitamin formulations was associated with a lower risk of AMD progression and is currently employed as the primary therapy in a subset of patients to reduce the progression of AMD. The current AREDS2 formula consists of zinc (80 mg), copper (2 mg), vitamin C (500 mg), vitamin E (180 mg), lutein (10 mg), and zeaxanthin (2 mg). Carotenoids in general (of which lutein and zeaxanthin are two that primarily contribute to the macular pigment) have been shown quench singlet oxygen, thereby preventing lipid peroxidation and upregulating antioxidant genes, including glutathione.[59]

Nicotinamide is a water-soluble form of vitamin B3 or niacin that is a component of NADH and NADPH and critical for ox-redox reactions such as glycolysis and ETC. In the eye, nicotinamide has been shown to be protective against consequences of mitochondrial dysfunction. Supplementation with nicotinamide decreased the development of glaucoma by ~10-fold in a mouse model of glaucoma.[60] Nicotinamide has also been shown to inhibit the expression of inflammatory and drusen-related genes in ARMS2/HTRA1

AMD-induced pluripotent stem cell–RPE cell lines.[61] Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is plant sterol found in many food sources that activates the sirtuin family of histone deacetylases (i.e., SIRT1) and adenosine monophosphate protein kinase (AMPK). While the specific mechanisms of action are debated and the effects are known, resveratrol has been shown to exhibit antiangiogenic, antioxidative, anti-inflammatory, and neuroprotective properties in RPE cell culture.[62]

To combat the oxidative stress and subsequent RGC death seen in glaucoma, a recent study found that intravitreal injection of adeno-associated virus–mediated superoxide dismutase (SOD) gene therapy significantly inhibited RGC death secondary to chronic intraocular pressure (IOP) elevation by enhancing antioxidative enzyme expression/activity and reducing lipid peroxidation.[63] Curcumin is an antioxidant agent found in turmeric that has been studied for LHON in a phase III clinical trial. In the treated group, glutathione peroxidase and SOD enzyme activities were decreased at 3 and 6 months after treatment, but visual results have not been released, and given the delay, it is likely that no visual improvement was achieved with curcumin (NCT00528151).[64] N-acetylcysteine, CoQ10, and dihydrolipoic acid have been shown to rescue mitochondrial respiration defects in fibroblasts from a patient with neuropathy, ataxia, and retinitis pigmentosa (NARP) syndrome.[65] NARP is caused by a missense mutation in mtDNA gene coding for ATPase 6, complex V subunit. Clinical manifestations are complex and heterogeneous with salt and pepper retinopathy, retinitis pigmentosa, sluggish pupils, proximal muscle weakness, hearing loss, dementia, seizures, and ataxia.[66]

Serotonin receptor agonists (5-hydroxy-tryptamine 1a) have also been shown to induce antioxidant protection through enzyme expression and preserve retinal structure in function in a mouse model of chronic mitochondrial oxidative stress.[67] Many other antioxidants, including melatonin,[68] glutathione S-transferase,[69] N-tert-butyl hydroxylamine,[70] α crystallins,[71] and L-carnitine,[72] have been explored as well.

Mitochondrial Quality Control (Fusion/Fission, Biogenesis/Mitophagy)

Although mitochondria have historically been largely regarded as static bean-shaped organelles, driven by observations on electron micrographs, the advent of newer imaging techniques, such as fluorescent microscopy and fluorescent molecule/protein labeling, has led us to appreciate that mitochondria are dynamically interconnected and capable of changing size, numbers, and position within cells on second/minute time scales (Fig. 1C). Mitochondrial interconnectivity is controlled in part by *fusion*, the joining of mitochondria to form one mitochondrion, or by *fission*, the budding of mitochondria to form two or more distinct mitochondria.[73] This activity also influences mitochondrial density (i.e., increased fission activity can produce smaller and more numerous mitochondria or increased fusion, lending to larger networked mitochondria within a particular cellular region). Alternatively, the number of mitochondria in a cell can also gradually increase through the production mitochondrial components, resulting in the synthesis of new mitochondria termed *mitochondrial biogenesis*. The alternative to this process is the destruction of mitochondria through a process termed *mitophagy*, regulated in a large part by autophagosome/mitophagosome-based degradation of mitochondria.[74] Importantly, all these activities are critical for "mitochondrial quality control," clearing dysfunctional mitochondria by mitophagy, adding new healthy mitochondria by biogenesis, and dispersing defective mitochondrial proteins for later repair or isolating unrepairable mitochondria for subsequent mitophagy through fusion-fission, respectively.[75] Thus, each of these activities works in concert with the other to ultimately stabilize mitochondrial function, and therapeutics that can manipulate these functions to guide mitochondria toward a more functional status in disease can benefit cellular health and possible reverse vision loss.

Fusion

Mitochondrial fusion and fission are required to maintain mitochondrial function. To coordinate these opposing events, a discrete set of nuclear encoded proteins is shuttled to the mitochondrial membrane. Fusion is mainly regulated by three proteins that function through GTPase activity to join the outer mitochondrial membranes of adjacent mitochondria through mitofusin 1 and mitofusin 2 activity, followed by inner membrane fusion through optic atrophy 1 (OPA1) activity.[76] The latter protein is coded for by one of the main genes involved in ADOA, a condition that leads to progressive RGC loss as previously described. Mitofusins have also been linked with optic atrophy, as evidence points to the causative role of genetic mutations

in *MFN2* in RGC axonal degeneration in association with Charcot-Marie-Tooth type 2A peripheral neuropathies,[77] suggesting a protective role of fusion activity. In addition, increased fusion/suppressing fission activity is thought to promote neuroprotection and mitigate damage in nongenetic conditions, as after glaucomatous injury. Despite mounting evidence for the efficacy of targeting fusion activity in the setting of injury or disease in the visual system, particularly as it relates to neuro-ophthalmic conditions, few fusion-promoting drugs have been tested in the eye. Thus, it will be important to repurpose compounds that have agonistic effects on fusion for use in visual system diseases or injuries, such as hydroxylamine derivative *BGP-15* (O-[3-piperidino-2-hydroxy-1-propyl]-nicotinic amidoxime), an OPA1 activator[78]; *chimera B-A/l*, an MFN activator[79]; and *leflunomide*, a novel MFN1/2 protein production stimulating compound.[80]

Fission

A key protein regulating mitochondrial fission is dynamin-related protein 1 (DRP1), a GTPase that squeezes and severs the outer membrane of mitochondria, producing multiple and smaller counterpart intact mitochondria.[76] Mutations in DRP1 have yet to be labeled as causative in genetic diseases of the visual system, possibly due to the frequent degree of embryonic lethality in association with loss of function of DRP1.[81] However, increased levels of DRP1 were found in retinas of glaucomatous mice as well as in cultured RGCs exposed to elevated hydrostatic pressure in vitro.[82] In addition, injuries of the visual system are known to have an acute fission response preceding apoptotic events It has also been shown that DRP1 inhibition, by a compound called *Mdivi-1*, ameliorated oxidative stress–mediated mitochondrial fission, RGC dysfunction, and cell death in a glaucoma mouse model, providing evidence for the clinical utility of targeting fission in neuro-ophthalmic disease.[83] However, like the fusion-modifying drugs, few other fission-modifying compounds have been developed and tested within the visual system, providing an opportunity for future drug development and repurposing withing the ophthalmic disease/dysfunction space.

Biogenesis

Mitochondrial biogenesis is an orchestrated control of the number of mitochondria per cell through a complex set of signaling pathways, transcriptional activity, and the shuttling of proteins between the nucleus and mitochondria. The central regulator for the signaling pathways that trigger mitochondrial biogenesis is the transcriptional coactivator *proliferator-activated receptor gamma coactivator 1 alpha* (*PGC1α*), which acts as an intermediator between numerous and yet to be fully elucidated signaling pathways.[84,85] Upon activation, PGC1α translocates to the nucleus and acts as a coactivator of transcription factors, such as nuclear respiratory factor 1 and nuclear receptor transcription factor. This transcriptional activity leads to the production of transcripts involved in oxidative phosphorylation and increased mtDNA replication, such as mitochondrial transcription factor A (TFAM). The final result is an increase in nuclear and mitochondrial encoded mitochondrial proteins that, through interacting processes of fission/fusion and mitophagy, leads to mitochondrial turnover and/or increases in mitochondria area/number in a cell (Fig. 1C).

It is now appreciated that a broad range of compounds can upregulate mitochondrial biogenesis. Activating PGC1α and PGC1β may help prevent retinal degeneration in AMD.[86] *Resveratrol* and other SIRT1 activators, also known for their antioxidant properties, have been shown to upregulate PGC1α and increase mitochondrial activity and area in multiple cell types.[87–90] Increased PGC1α activity/expression, mtDNA expression, and/or TFAM expression has also been documented with the adenosine monophosphate-activated kinase activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), G protein coupled receptor agonists (formoterol, ly344864, DOI, and rimonabant), nuclear receptor agonists (fibrates, thiazolidinediones, GW501516, and dexamethasone), cGMP modulators (sildenafil, vardenafil, DETA-NO, Bay 41-2272, cinaciguat, and riociguat), and natural products (green tea polyphenols, lipoamide, isoflavones, and quercetin), reviewed in greater detail by Whitaker et al.[84]

Relevant to ocular disease, circulating estrogens have also been shown to activate mitochondrial biogenesis in cells carrying an LHON mutation, stimulating an increase in PGC1α expression, increased mitochondrial density, reduced ROS levels, and reduced cell death.[91,92] The findings in this LHON study also set a precedent for future investigation, which could apply the above highlighted as well as an expanded list of biogenesis-regulating compounds toward broadly treating ophthalmology diseases that are caused by or have associate mitochondrial dysfunction.

Mitophagy

Mitophagy is the selective degradation and recycling of old and dysfunctional mitochondria through a process that involves sequestering mitochon-

dria to a lipid membrane, called the mitophagasome, and trafficking to lysosomes for destruction. This process parallels autophagy and is often conceptually blurred as both processes engulf mitochondria with the exception that mitophagy is thought to exclude damaged cytosolic proteins and other organelles and is thought to specifically regulate dysfunctional mitochondria.[74] Regardless, autophagy/mitophagy can participate in mitochondrial "cleanup," which is ultimately essential to proper cellular function and energy production. Central to this process are the cytosolic proteins PTEN induced kinase 1 (PINK1) and E3 ubiquitin ligase parkin (Parkin),[93] which translocate to the outer mitochondrial membrane to mark and engage the process of degradation of damaged and often hyper-fizzed mitochondria.[94] However, as with other dynamic mitochondrial activities, there seems to be other inputs onto PINK1/Parkin and the activation of mitophagy. Central to the identification of these inputs and potential pharmacologic targets are conditions in which mitophagy/autophagy has been shown to be a significant pathophysiologic mechanism of disease such as AMD,[95] diabetic retinopathy,[96] retinal degenerative disease, glaucoma, and cataract.[97]

Sirolimus (rapamycin) is a potent inducer of autophagy and activator of the mTOR pathway that has been used in ocular inflammatory disease.[98] Despite prior positive reports of the therapeutic benefit of sirolimus in nonexudative AMD, a recent prospective randomized study found that intravitreal sirolimus failed to halt the progression of geographic atrophy compared to sham treatment.[99] Metformin, a commonly used oral therapy for diabetes, has also been shown to act on mitophagy pathways. Metformin lowers blood glucose levels by decreasing hepatic glucose production, increasing insulin sensitivity, and slowing intestinal glucose absorption. In a sense, metformin simulates caloric restriction and cellular "starvation," which may lend to its therapeutic properties. Although all of metformin's mechanism(s) are not fully understood, it is known that metformin improves mitophagy activating AMPK via phosphorylation[100] and decreasing cytosolic p53.[101] Metformin has been shown to suppress retinal angiogenesis and inflammation seen in diabetic retinopathy[102] and may have a protective effect against the development of nonexudative AMD.[103] Additional AMPK agonists include AICAR,[104] coenzyme Q, and compounds that allosterically activate AMPK in an AMP-independent manner such as PT1, shown to protect against photoreceptor and RPE loss in a mouse model of retinal degeneration.[105] Notoginsenoside R1 has also been shown to reduce retinopathy within a mouse model of diabetic retinopathy through activation mitophagy via the PINK1/Parkin pathway.[106]

Additional drugs acting on mitophagy pathways have been used in other disease scenarios and may show promise in the eye. Mito-targeted 3-carboxyl proxyl nitroxide has been shown to induce mitophagy in colon cancer models.[107] Urolithin A, an activator of mitophagy, was found in a phase I clinical trial to be safe in elderly patients and shown to increase mitochondrial gene expression in skeletal muscle.[108] Gerontoxanthone I and macularxanthone were also shown to induce PINK1/Parkin-mediated mitophagy in an myocardial ischemia/reperfusion injury model.[109] These and other mitophagy inducing compounds show promise in the treatment of both inherited and acquired disease, but many questions remain.

Apoptosis

When all cellular and mitochondrial quality control and/or ROS reducing mechanisms fail to restore or remove defective mitochondria, cells can activate a self-destruct signaling mechanism known as apoptosis.[110] Whether triggered by cellular or mitochondrial dysfunction, apoptosis signaling is partially dependent on the release of the mitochondrial ETC hemeprotein cytochrome c, ultimately leading to the activations of proteases that degrade a cell from within.[111] This self-destruct mechanism generally ensures the clearance of defective cells, thus preserving the larger organ function. However, in organs where some cells fail to turn over, such as within the visual system, this apoptotic process may lead to irreversible loss of function.[112] As a consequence, many scientific efforts have focused on identifying therapeutics that can slow or halt the process of irreversible vision loss by targeting the reduction in apoptotic activity triggered by diseases or injury.

While many studies have shown that drugs of various classes have antiapoptotic activity, few of these drugs have been directly tested in human visual diseases. Among those that have been evaluated is the mitochondrial-derived peptide, humanin, which has been shown to be cytoprotective in numerous neurodegenerative disease and showed antiapoptotic activity in cybrid cell lines carrying mitochondria from patients with AMD.[113] Interestingly, cybrid cell lines carrying transplanted mitochondria from patients with AMD experience reduced levels of cell viability, mtDNA copy numbers, mitochondrial replication/transcription genes, and antioxidant enzyme genes, along with elevated levels apoptotic genes and mtDNA fragmenta-

tion.[113] Overall, this further supports a mitochondrial-centered pathophysiology in AMD and highlights the importance of therapeutics targeting mitochondria to reduce the apoptotic burden of AMD.

Another compound with antiapoptotic properties is cyclosporin, a commonly used immunosuppressant that inhibits calcineurin. An ophthalmic emulsion of cyclosporin is commercially available (Restasis, cyclosporin 0.05%; Cequa, cyclosporin 0.09%) for selected patients with dry eye syndrome with significant inflammatory component.[114] Cyclosporin has also been shown to bind calcineurin B in the mitochondrial matrix and inhibit the opening of the mitochondrial permeability transition pore (MTPT), thereby preventing the entrance of calcium and release of cytochrome *c* to the cytosol involved in triggering cell death pathways.[115] These findings along with encouraging in vivo results from animal models[116] have led to the use cyclosporin for treating LHON. However, an open-label pilot study evaluating the efficacy of oral cyclosporin in preventing progression of the fellow eye in patients with LHON with early unilateral disease within 6 months of symptom onset failed to meet its primary endpoint, as by the end of the study, both eyes were equally affected.[117] The rationale of cyclosporin development and subsequent approval for dry eye syndrome is substantially based on its cyclophilin-mediated pathway. However, based on these recent findings, benefits from this drug can be partially due to the MTPT pathway as well, highlighting the role of mitochondrial dysfunction in dry eye and ocular surface disease.[118]

Mitochondrial Genetic-Based Therapies and Mitochondrial Replacement

Mitochondria have their own circular DNA (mtDNA), which contains 37 genes that encode for 13 protein subunits of the ETC, 2 subunits of mt-ribosomes, and 22 mt-tRNAs. Most proteins in mitochondria, however, are encoded by nuclear DNA (nDNA).[119] Therefore, at a gene level, mitochondrial disease can be classified according to whether the causal mutation occurs in nDNA or mtDNA. In addition, whether the causal mutation is nuclear or mitochondrial has implications for possible gene therapy approaches, highlighted here by four promising strategies that are in the clinical setting or have potential for clinical utility. (1) Traditional gene therapy, here referred to as approaches targeting the manipulation and/or expression of nuclear encoded mitochondrial genes by viral delivery of DNA or RNA (covered extensively elsewhere[120]), may be utilized if the mitochondrial disease is caused by nDNA mutations. However, when disease is caused by a mtDNA mutation, gene therapy is major challenge as mitochondria do not readily import DNA or RNA. To overcome this challenge, the following advances have enabled nucleic acid delivery to mitochondria. (2) Allotopic expression is a method to express modified mitochondrial encoded genes in the cytosol with a fused mitochondrial targeting sequence to facilitate mitochondrial import of the subsequently translated protein.[121] (3) mtDNA gene editing, like nDNA gene editing, allows the mutated sequence to be corrected.[122,123] Finally, (4) mitochondrial replacement therapy is being pursued as a specialized technique to prevent maternal transmission of disease altogether. These approaches remain experimental, but as discussed below, some have been applied to patients with LHON. Given that LHON mutations are mitochondrial encoded, we will focus mainly on the novel methods used for improving mtDNA encoded disease such as allotopic expression, with a brief review of mitochondrial gene editing and mitochondrial replacement therapy. Of note, while mitochondrial gene editing and mitochondrial replacement therapies have not been specifically applied for eye disease, the fact that mitochondrial DNA mutations represent a significant driver of eye disease[124] sets the precedence for the future of application of these approaches to treat eye disease and worth discussing here.

Allotopic Expression

In allotopic expression, there a few modifications that need to be applied to successfully express and deliver a mitochondrial encoded gene to the mitochondria. First, the mitochondrial encoded gene must be codon optimized for nuclear expression, as to accommodate for the differences in translation between the mitochondrial genome and nuclear genome.[125] Then, to deliver the now nuclear-expressed and cytoplasmic-translated mitochondrial encoded protein to mitochondria, a mitochondrial targeting sequence is fused to the gene. To deliver this modified mitochondrial gene, genetic payloads can be delivered by a viral vector or, in principle, be transfected directly to the cell as modified mRNA or protein. Allotopic expression was first applied in 2002 for the MT-ATP6 gene[126] responsible for NARP and in the same year for MT-ND4, responsible for LHON,[127] with both cases showing a significant improvement in in vitro ATP production. Then, in vivo studies in rodents showed

that intravitreal injections of the adeno-associated virus serotype 2 (AAV-2)–mediated MT-ND4 allotopic expression prevented RGC loss and improved visual function.[128,129] Ultimately, these findings paved the road for a series of human clinical trials using allotropic expression for treating LHON.

In two studies where patients with LHON, carrying the most common point mutation (m.11778G>A), were treated with allotopically expressed MT-ND4 delivered via AAV-2 intravitreal injections, two of five patients[130] and six of nine patients[131] experienced improvement in visual acuity, with no reported adverse effects. Another gene therapy trial for LHON with a longer follow-up (36 months) showed that unilateral intravitreal injection of AAV-2 allotically expressing MT-ND4 was safe and effective at improving BCVA and visual fields (VFs) in at least one eye in six of nine patients.[132] At 7 years, VA gains persisted in six of eight patients who remained in the study, and no adverse events were recorded.[133] Preliminary data from another phase II/III study, performed by the same group, showed that rapid response to treatment (defined as improvement of ≥0.3 LogMAR by day 3) was negatively correlated with duration of the disease, positively correlated with baseline BCVA, and independent from baseline VF and baseline retinal nerve fiber layer (RNFL) thickness.[13]

In parallel, beginning in 2015, GenSight Biologics sponsored multiple clinical trials to assess the safety and efficacy of a branded version of the AAV-2–mediated allotropical expression of MT-ND4 in patients with LHON, branded as GS010 (or lenadogene nolparvovec; Lumevoq, GenSight Biologics (Paris, France)). The phase I/II Safety Evaluation of Gene Therapy in LHON Patients (NCT02064569) study demonstrated its safety and tolerability at 48 weeks after an escalating dose of a single unilateral intravitreal injection in the worst eye. Reported adverse events were similar to previous studies on intravitreal injections, including substantially mild ocular inflammation and IOP elevation. Furthermore, at 96 weeks, significant BCVA improvement was noted in 6 of 14 patients, and treated eyes showed a different response of +14 Early Treatment Diabetic Retinopathy Study (ETDRS) letters compared to the fellow eyes only in the subgroup with onset ≤2 years and baseline BCVA ≥20/12,000.[134]

The Efficacy Study of GS010 for the Treatment of Vision Loss up to 6 Months from Onset in LHON Due to the ND4 Mutation (RESCUE[135]; NCT02652767) and Efficacy Study of GS010 for Treatment of Vision Loss from 7 Months to 1 Year from Onset in LHON Due to the ND4 Mutation (REVERSE[136]; NCT02652780) are two parallel studies that compared

BCVA (considered significant when difference ≤0.3 LogMAR or ≥15 ETDRS letters) of GS010-treated eyes to fellow sham-treated eyes at 48 weeks after intravitreal injection in patients with onset of the disease within 6 months (RESCUE) and those with onset between 7 and 12 months (REVERSE) as the primary outcome. In both studies, BCVA deteriorated similarly in both eyes, reaching the nadir around 30 to 40 weeks from the onset of vision loss followed by improvement. Unexpectedly, visual outcomes of the fellow eye showed a similar trend, and therefore both studies did not meet their primary endpoint (difference of –0.3 LogMAR between the treated and untreated eye). Bilateral visual recovery began on average 24 and 12 weeks after injection in RESCUE and REVERSE studies, respectively. Another unexpected and unintuitive finding that came out by comparing the two studies is that patients treated in later stages in the REVERSE study achieved better visual outcomes and earlier compared to those treated in earlier stages in the RESCUE study, even when the data were normalized by onset of vision loss. The authors speculated about the possible effect of RNFL thickness in the initial stages being a physical barrier to the diffusion of the drug. Both studies showed no serious adverse events related to the study drug; the most frequent side effects reported were mild or moderate intraocular inflammation (74%–92%) and an increase in IOP (27%–33%). At 2 weeks posttreatment, viral vector biodissemination was assessed, and among the two cohorts of 39 and 37 patients, only 2 were positive for viral DNA.[135]

An interim analysis of the natural history data of untreated patients derived by the cross-sectional Observational Registry Study of LHON Affected Patients (REALITY; NCT03295071) showed that after an initial rapid decline of vision, the progression slows down but with a progressive deterioration. These data are in accordance with a recent meta-analysis on the natural history of LHON.[137] Even though both RESCUE and REVERSE trials failed, when longer-term data are compared with preliminary results from the REALITY study, GS010 was able to restore vision significantly compared to the nadir. In addition, 94 weeks of data from the RESCUE and REVERSE studies kept showing a better visual trend for the treated eye even though it did not reach statistical significance.[135,136]

The Efficacy & Safety Study of Bilateral IVT Injection of GS010 in LHON Subjects Due to the ND4 Mutation for up to 1 Year (REFLECT; NCT03293524) is the first randomized double-masked placebo-controlled trial evaluating the safety and efficacy of bilateral intravitreal injection of GS010 in patients with LHON with onset within 1 year. This

study is active but not yet recruiting and will compare the change of BCVA at 12 months posttreatment in the least affected eye.

Overall, these findings add strong support for the use of gene therapy and allotopic expression in treating mitochondrial encoded diseases. However, it will be important to know if this approach can be broadly applied to patients with mitochondrial disease, as future efforts will need to demonstrate enhanced mitochondrial function and mitigation of vision loss in patients experiencing other mutations and mitochondrial diseases beyond LHON.

mtDNA Editing

Mitochondrial gene editing is made difficult by the fact that mitochondria do not readily import RNA, making gene editing with current clustered regularly interspaced short palindromic repeats (CRISPR)-based systems that utilize a guide RNA ineffective. However, two experimental approaches have emerged to overcome this problem: the application of mitochondrially targeted transcription activator-like effector nucleases (mitoTALENs) and DddA-derived cytosine base editor.[122,123] Although these approaches have yet to be applied in a clinical trial setting, the evidence provided by the laboratory studies suggests an optimized application for different mitochondrial disease paradigms.

For example, mitoTALENs, which are proteins designed to be mitochondrial targeted, are used to selectively bind mtDNA and produce double-strand breaks through nuclease activity. Given the lack of efficient repair mechanisms for double-strand breaks in mtDNA,[138] mitoTALENs designed to target mtDNA mutations can lead to a clearance of deleterious mtDNA from mitochondria.[139] Thus, in heteroplasmic diseases such as Kearns–Sayre syndrome[140] or MELAS,[141] mitoTALENs are well suited to clear out unwanted mtDNA, as demonstrated both in vitro and in vivo in mouse models of mitochondrial disease using AAV delivery to heart and skeletal muscle tissue.[142] However, when mtDNA mutations are homoplasmic or comprising the majority of mtDNA, such as with most LHON cases,[143] the applications of mitoTAL-ENs could lead to an overall depravation of mtDNA from cells. Consequently, the clearance of mutations in homoplasmic disease would potentially lead to more harm than benefit from mitoTALEN interventions, thus setting the premise for therapies that focus on correcting mtDNA mutations rather than clearing them.

A recent study has provided some evidence that the challenges of mtDNA editing can be overcome with an RNA free application, combining the mtDNA target-ing potential of transcription activator-like effector proteins with a base editing enzyme. This approach[123] utilizes a cytidine deaminase enzyme named DddA to catalyze a C-G pairing to a T-A pairing in double-stranded DNA. Thus, by fusing this enzyme with a mitochondrial targeted TALE, mtDNA genes carrying deleterious point mutations, in which a C/G has been substituted for a T/A, can now be corrected back to the T/A carrying wild-type gene. A prime example where this can be applied is with the common LHON mutation m.14484T>C, which has been identified in homoplasmic or high heteroplasmic states.[144] Thus, applying DddA to patients with LHON or patients who have high levels of mutated mtDNA might be an elegant solution to overcome the limitations of mtDNA depletion approaches. Of course, this mitochondrial editing approach will only be applicable to mutations resulting in a C or G and not mutations resulting in an A or a T, and there can be difficulty targeting certain regions within the mitochondrial genome due to TALEN design challenges. Furthermore, the mitochondrial gene-editing potential of Ddda has only been demonstrated in vitro in HeLa and has shown at best a 50% efficiency toward editing a specific mitochondrial target,[126] thus presenting a level of uncertainty as to whether this approach can provide an efficacious change in mitochondrial disease models and, more important, affected individuals. Still, this is the first gene editing approach of its kind and presents a substantial leap forward in gene editing of mitochondrial encoded mutations, worthy of future pursuit toward clinical utility. Ultimately, both mitoTALENs and mitochondrial targeted DddA-derived cytosine base editors present a bright future for mitochondrial disease therapy, and the ultimate clinical utility is uncertain given that neither of these approaches has been tested in for efficacy in human subjects.

Mitochondrial Transfer Therapies

As highlighted above, gene therapy shows promising benefit for improving quality of life for patients carrying mtDNA mutations by supporting different organ systems, especially within the ophthalmology field. However, for families burdened with vision and other systemic dysfunction from mitochondrial disease and who want to conceive children and reduce the potential for heritable mitochondrial eye disease,[6] gene therapy is still rather limited and not yet approved for reproductive purposes. Thus, some patients who want to have healthy genetic offspring have turned to reproductive medicine techniques to avoid passing down the debilitating and sometimes life-shortening burdens of mitochondrial disease. Referred to as *mitochon-*

drial replacement therapy, this reproductive therapy entails the transfer of pronuclei in fertilized zygotes or the mitotic spindle in unfertilized oocytes from a female patient carrying mtDNA disease into an enucleated zygote or oocyte from a healthy donor, creating a cybrid oocyte with a disease-free nuclear genome from the patient and functional mitochondria from the donor.[145,146]

Pioneered in the mid-1990s, early approaches were based on mitochondrial transfer technology developed to treat infertility in aging women, in which healthy mitochondria along with cytoplasm were pulled from donor oocytes and directly injected into oocytes from an infertile patient, termed *cytoplasmic transfer*.[147] This resulted in the birth of the first child with DNA from three different parents and was deemed an experimental approach for infertility for older patients. However, this approach has inherent risk for patients with mitochondrial disease, as the transferred healthy mitochondria would only dilute the mitochondria carrying deleterious mtDNA. In addition, there is the risk of potentially carrying over chromosomal material, allowing an oocyte to come to term that otherwise may not be viable due to disease burden and/or inadvertently introduced chromosomal material. Despite these potential drawbacks, these studies sparked research into the modern approach of spindle/pronuclear transfers in monkeys,[148] followed by the first human birth in 2016 to receive a spindle transfer.[149] This patient was carrying the m.8993T>G mutation, known to affect the mtDNA-encoded ATPase gene (MT-ATP6) causing Leigh syndrome, a disease associated with debilitating neurologic symptoms and retinopathies.[150] The baby was born healthy; however, long-term follow-up is needed to evaluate the complete safety of this approach. Nonetheless, in 2016, at the request of the FDA, the National Academies of Sciences, Engineering, and Medicine deemed the use of mitochondrial transfer therapy ethically permissible but only for those at risk of transmitting mitochondrial disease and in male offspring, to avoid potential propagation of mitochondrial disease and the unknown consequence of mitochondrial replacement therapy.[151] In 2017, the National Health Service in the United Kingdom granted the first license to perform this procedure to the Newcastle Fertility Center,[145] setting the precedent for other countries to follow in the future. Thus, while there are some concerns as to the future consequences of mitochondrial transfer therapy, this approach offers an exciting avenue for mothers diagnosed with mitochondrial disease who want to avoid maternal transfer of deleterious mtDNA to their offspring.

Conclusion

Although most mitochondrial disorders are multisystemic, eye involvement occurs commonly and is often disabling. A more comprehensive understanding of basic mitochondrial biology and disease pathogenesis has led to the development of multiple potential therapeutic approaches, each targeting a specific area of mitochondrial biology. Although primary mitochondrial eye disease caused by specific genetic mutations is relatively rare, secondary mitochondrial involvement in common eye conditions, such as those related to aging, may also be responsive to successful treatments developed for genetic mitochondrial disorders. Therefore, a mitochondrial therapy developed for a specific genetic disease may have widespread implications for the treatment of a variety of conditions that affect the eye. Various methods of delivery for the therapies discussed above have been proposed, including topical, intracameral, intravitreal, subretinal, suprachoroidal, periocular, and systemic, but further investigation is necessary to determine the safest and most efficacious method for mitochondrial therapeutics.[152] As mitochondrial therapies continue to progress, early disease diagnosis and timely therapeutic intervention will be critically important. Despite the current lack of an FDA-approved treatment for mitochondrial disease, the translation of scientific knowledge into practical therapies for ophthalmology patients affected by mitochondrial dysfunction continues to advance.

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