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GENE THERAPIES FOR EYE DISEASES

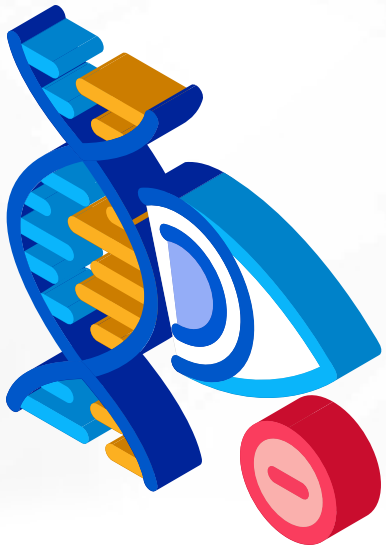
INTRODUCTION

Gene therapy approaches to treat eye diseases have been rapidly evolving, and the rapid rate of progress suggests that gene therapy has a bright future to address genetic or inherited eye diseases. Applying gene therapies to the eye has unique advantages in that the eye is compartmentalized and easily accessible for drug administration and subsequent prognostic monitoring. The eye is also immune-privileged so introducing viral vectors or cell therapies does not induce an inflammatory response. Delivering gene therapies directly to the eye via localized injection is an efficient method to target the cells of interest. Some of the localized routes of administration include retro-orbital, intravitreal or subretinal injection. An added benefit of efficient drug delivery is manageable dosing of viral vectors compared to systemic delivery where higher viral doses are needed to achieve therapeutic efficacy. Due to these advantages, gene therapy approaches are now being developed as curative therapies for genetic vision loss.

There has been strong progress in the preclinical identification and validation of gene therapies. The majority of genes involved in eye development, function and maintenance have been identified, and mutations in some of these genes have been shown to be disease drivers of ocular diseases. One example is the presence of mutations in the *RPE65* gene that is reported to cause various forms of retinitis pigmentosa and Leber's congenital amaurosis.

Several eye diseases are being modeled in mice and the development and characterization of mouse models for various eye diseases including vascular eye diseases, nonsyndromic and syndromic eye diseases, retinal diseases etc. have been widely published[1]. Developing a mouse model for an eye disease allows noninvasive screening of therapies and allows the rapid identification of disease morphology. However, there are significant differences between mouse and human eyes in terms of cell composition and receptor density, so increasingly large animal models are being developed to study specific eye diseases. For example, transgenic pig models are being used to study retinal dystrophies and Stargardt-like macular dystrophy type 3 while dog models are used to study retinal dystrophies and cone dystrophies[2]. The development of targeted gene therapies that are validated in improved animal models will help expedite the movement of therapies into the clinic.

APPROVED THERAPIES

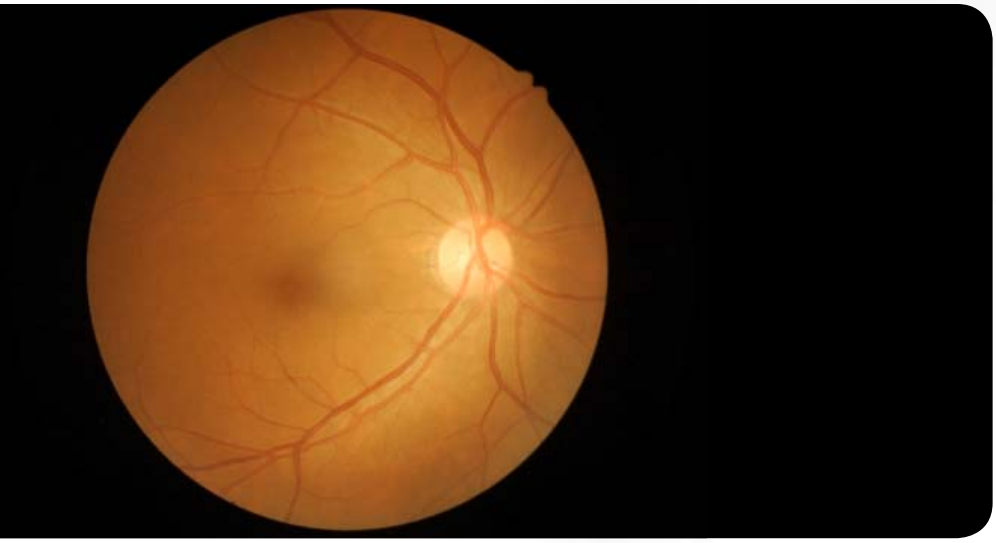


The first gene therapy approved to treat genetic diseases was Luxturna®[3, 4], an adeno-associated virus (AAV) therapy that is injected subretinally to treat inherited retinal diseases such as Leber's congenital amaurosis (LCA) or retinitis pigmentosa (RP). Luxturna can be used to treat patient who have mutations in both copies of the RPE65 gene, as it is an AAV vector that carries a functional copy of the *RPE65* gene. The RPE65 protein is necessary to convert light entering the eye into electrical impulses that are transmitted to the brain.

The *RPE65* gene therapy has paved the way for gene therapies to treat other eye diseases including, age-related macular degeneration, choroideremia and other diseases[3, 5]. Administering AAVs carrying replacement genes via direct injection into the eye is considered to be an efficacious and safe route of administration but one of the key limitations is small packaging capacity. AAVs have small genomes so the payload gene has to be about 3 to 4 kb. This can limit vector design to target specific diseases such as Stargardt disease where the *ABCA4* cDNA is 6.8 kb. Lentiviruses are an alternative to package larger genes that are in the 5-6 kb range.

CURRENT CLINICAL APPROACHES

Over 60 interventional clinical trials for gene therapies in ocular disease are currently ongoing with about two-thirds of the trials in phase I or II[6, 7]. Most of the trials use AAV as a delivery system since it has a good safety profile. Treatment of specific ocular diseases using gene therapies are being investigated. One of the disease areas of interest is neovascular or "wet" age related macular degeneration (AMD), where inhibition of abnormal blood vessel formation or growth are the key endpoint. Retinostat is a lentiviral therapy delivering two anti-angiogenic genes (endostatin and angiostatin) that is being tested in neovascular AMD[8]. Another therapy that is being tested for wet AMD is an AAV virus carrying Flt-1 which is a known blocker of vascular endothelial growth factor (VEGF) and prevents new blood vessel formation[3]. In contrast, gene therapy approaches for "dry" AMD are not well advanced perhaps due to disease complexity and the lack of a clearly druggable target[9].



Stem cell and non-stem cell therapies are being tested for AMD. Palucorcel from Janssen Pharmaceuticals is a non-stem cell therapy derived from human umbilical tissues that has shown promising preclinical data and phase I data. A phase II trial on 21 AMD patients was completed in November 2019. Similarly, a phase I/II trial on advanced dry AMD patients using a novel cell therapy MA09-hRPE is ongoing. This cell therapy approach uses retinal pigmented cells derived from human embryonic stem cells (hESCs).

The success of Luxturna has spurred the testing of hRPE65 gene therapy in other diseases such as retinal dystrophy due to *RPE65* gene mutations. Other areas where gene therapy approaches are being evaluated include retinoblastoma where an oncolytic adenovirus (VCN-01) is being tested in a phase I trial[6] and choroideremia where an AAV based vector expressing REP1 is being evaluated. REP1 is involved in the lipid modification of Rab GTPases. REP1 and REP2 are highly homologous proteins and REP2 is hypothesized to compensate for loss of REP1 expression in other cells except the eye, which is the rationale behind introducing a functional copy of the REP1 gene in the eye using the AAV vector[10].

PRECLINICAL GENE THERAPY EXAMPLES

Gene therapy for Stargardt disease

Stargardt disease is a monogenic inherited disorder of the retina that causes vision loss. Stargardt disease is the most commonly inherited pediatric and adult maculopathy and can present during childhood or adulthood. The disease is characterized by macular degeneration and cells that lack the ABCA4 protein accumulate lipofuscin clumps in the retinal pigment epithelial cells. These clumps are visible as yellow flecks in the eye. As the lipofuscin clumps increase, central vision becomes impaired leading to the death of photoreceptors and further visual impairment. Mutations in the *ABCA4* gene are considered to be the primary disease drivers since up to 95% of all Stargardt patients have a mutation in the *ABCA4* gene[11].

Gene therapy is a promising approach for monogenic retinal diseases such as Stargardt disease but one of the limitations with a viral approach is packaging size as the ABCA4 gene is too large to fit in a single AAV vector[12].

One approach has been to use dual AAV vectors to deliver two halves of the *ABCA4* gene but the challenge is low transduction efficiency and the expression of truncated nonfunctional ABCA4 protein. Lentiviruses have the packaging capacity to deliver the intact *ABCA4* gene and subretinal injection of *ABCA4* carrying lentivirus has shown efficacy. However, the concern with lentiviruses is genomic integration and the triggering of carcinongenesis.

A non-viral gene delivery approach such as nanoparticles is a viable option and PEGylated polylysine DNA nanoparticles have been used to deliver the *ABCA4* gene under the control of an eye-specific promoter[12]. Another approach is to use lipofectamine DNA nanoparticles. However, the non-viral gene delivery system has limitations to overcome cellular barriers and maintain prolonged *ABCA4* protein expression.

Currently, there are 2 therapies in clinical trials that are not based on gene therapy[6]. Emixustat is a RPE65 inhibitor developed by Acucela that is in Phase 3 clinical trials and acts by inhibiting RPE65. The other therapy is ALK-001 from Alkeus Pharmaceuticals that acts by inhibiting the formation of toxic vitamin A dimers in the photoreceptors.

Optogenetics therapy for Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a group of hereditary retinal diseases and is the leading cause of inherited blindness. It is estimated that 1.5 million people worldwide have the disease. There are no curative therapies for RP so the only therapeutic options are acetazolamide to reduce swelling and high doses of vitamin A to slow vision loss. Some RP patients may be eligible for retina implants or "bionic eyes" where an electrode array is attached to the retinal surface via surgery. However, such devices typically provide limited restoration of vision due to a limitation of the number of electrodes that are stand-ins for photoreceptor cells[13].

Optogenetics is a technique where light responsive opsin proteins are expressed in the cells of interest. Once opsins are expressed in the target cells, the cells can be functionally stimulated using light of specific wavelengths. Opsin proteins are light sensors and one of the more well studied opsin is channelrhodopsin-2 (ChR2) isolated from *Chlamydomonas reihardtii*. In the retina, there are several approaches to reactivate retinal circuits after introducing an opsin protein[14]. ChR2 can be expressed in all retinal cell types using a ubiquitous promoter or can be restricted to a few cell types. Introducing ChR2 or other opsins using AAV or other viral vectors has made it possible to reactivate light-insensitive retinas with the support of medical devices. A combination of viral vector and medical device is being currently tested in a phase I/II clinical trials. GS030 from Gensight Biologics has 2 components – an engineered AAV2 encoding a light activable channelrhodopsin protein that is administered intravitreally and biomimetic goggles that stimulate the engineered retinal cells by projecting images onto the retina by a light of specific wavelength[15].

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