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Title: GENE THERAPY IN RETINAL DISEASES
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Learning objectives:

1. Provide a history of gene therapy in regards to ocular conditions
2. Review gene therapy clinical trials
3. Describe different approaches to gene therapy
4. Explain the different methods of delivery of gene therapy
5. Discuss challenges with gene therapy

Key words**: Retinal disease, in vivo gene therapy, Luxturna, CRISPR**

GENE THERAPY IN RETINAL DISEASES

Introduction

Gene therapy delivers therapeutic nucleic acid material to targeted cells in order to modify the production of defective or deficient proteins.[1] The first therapeutic use of gene transfer was performed in 1990 at the National Institutes of Health (NIH), when four-year-old Ashanti DeSilva received treatment for a genetic defect that caused ADA-SCID, a severe immune system deficiency.[2] Between 1989 and 2018, over 2,900 gene therapy clinical trials have been conducted, with more than half of them in phase I.[3] However, none reached widespread use or received FDA approval.

The eye is an ideal target for gene therapy, as it serves as an immune-privileged and easily accessible site.[3] Modifications to entire genetic sequences have minimal side effects elsewhere in the body. Not surprisingly, the first US Food and Drug Administration (FDA)-approved gene therapy to reach the market was for treatment of an ocular disease. In 2017, Spark Therapeutics' Luxturna (voretigene neparvovec-rzyl) became the first ever in vivo gene therapy

approved by the FDA.[4-6] Used for the treatment of biallelic *RPE65* mutation-associated retinal dystrophy, Luxturna is an adeno-associated virus vector-based gene therapy that is injected into the subretinal space on a one-time basis. While not a cure for the condition, it has been shown to substantially improve vision in treated patients as measured by significant improvements in dim light navigation, full-field light sensitivity threshold, and visual fields.[4] Patients with pathogenic variants in both *RPE65* alleles typically present clinically with autosomal recessive Leber congenital amaurosis (LCA) or retinitis pigmentosa (RP). Vision loss is progressive and may become severely limited beginning in early childhood. The *RPE65* gene is expressed in retinal pigment epithelial (RPE) cells and is one of the critical enzymes involved in the visual cycle.

Like the studies that led to Luxturna, several gene therapy trials in ophthalmology have centered on monogenetic inherited retinal diseases (IRDs). IRDs are attractive targets for several reasons, including precise identification of a single causative gene and a more streamlined path to FDA approval with orphan disease status. An orphan disease is defined as a disease affecting less than 200,000 individuals, which applies to many IRDs.[1,7] However, the reach of these treatments is limited in scope, given the rarity of such conditions. Gene therapy to tackle more prevalent acquired conditions, such as age-related macular degeneration (AMD) and diabetic retinopathy, is far more challenging but would be able benefit millions of patients with retinal disease.

General Approaches to Gene Therapy

Gene therapies differ based on the inserted genetic material, the vehicle of delivery, and the route of administration to target cells.[8] The type of gene therapy utilized will depend on the particular disease's pathogenesis.

The following are various ways to modify a genetic defect:

1. Gene augmentation: this method aims to provide genetic material that will go on to produce functional protein that will replace what was lost in the targeted cells. This is typically used in autosomal recessive, loss-of-function IRDs, including Luxturna for *RPE65*-mediated retinal dystrophy.
2. Gene inactivation: this method aims to block production of an abnormal protein in the target cell. It may need to be combined with gene augmentation to restore production of a functional protein. This technique is useful for gain-of-function IRDs.
3. Gene editing: this method employs a "cut" and "paste" function to remove and then replace damaged DNA. The technology called CRISPR (clustered regularly interspaced short palindromic repeats) is currently available for gene editing, in which RNA coupled with the enzyme Cas9 identifies specific DNA sequences for excision. Although CRISPR is an exciting technology with potential widespread uses, the unintended side effects of CRISPR enzymes on non-target DNA sequences as well as the creation of new mutations calls for caution.

Regarding the method of delivery, gene therapy products can be given into the vitreous cavity or the subretinal space. Intravitreal injection is the least invasive method but does not ensure direct delivery of the gene therapy products to the targeted cells. This may lead to less efficacy or increased negative side effects. The subretinal space can be accessed either transretinally in conjunction with pars plana vitrectomy (**Figure 1**) or via the suprachoroidal space without a prerequisite vitrectomy (**Figure 2**). Subretinal injections are currently the most commonly employed technique for retinal gene therapy. Both approaches must be performed by a trained vitreoretinal surgeon who is well-versed in such techniques.

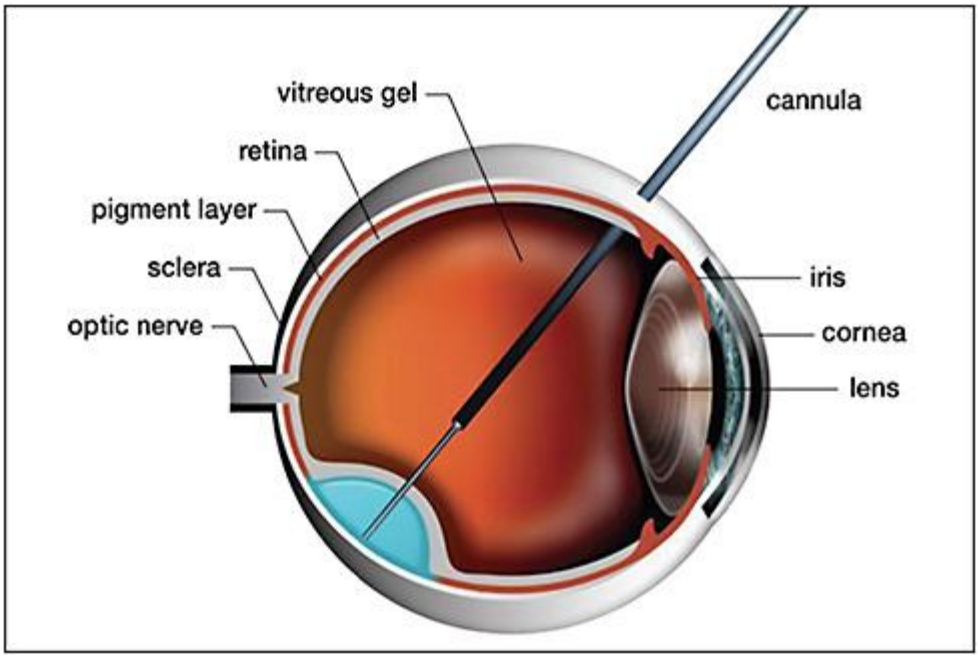

Figure 1. A fine microneedle is used to enter the subretinal space and inject gene therapy products directly under the damaged retinal cells, including the macula. IMAGE COURTESY OF MOORFIELD'S EYE HOSPITAL

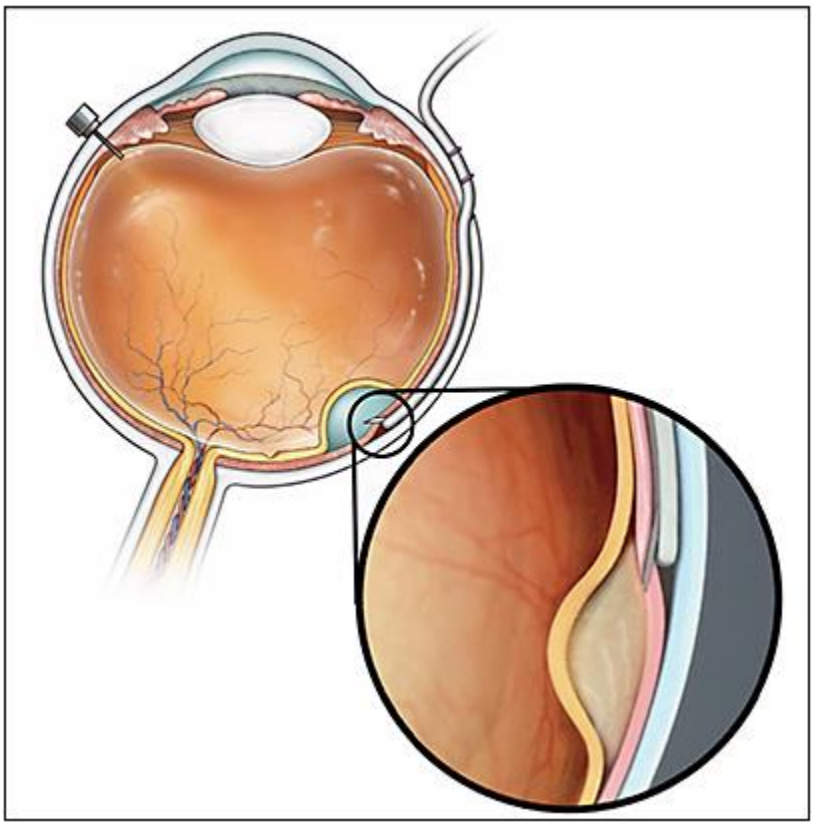

Figure 2. A catheter is threaded into the suprachoroidal space until it reaches the targeted region, at which point the gene therapy products are injected directly via a microneedle into the subretinal space. Featured here is the Orbit Subretinal Delivery System (Orbit SDS; Gyroscope Therapeutics). IMAGE COURTESY OF GYROSCOPE THERAPEUTICS

There are several clinical trials underway to treat the following IRDs using gene therapy: choroideremia, retinoschisis, X-linked retinoschisis, achromatopsia, Stargardt, and Usher syndrome.

In addition to these rare conditions, multiple clinical trials are currently being conducted for neovascular (wet) AMD, as well as one for non-neovascular (dry) AMD. Intravitreal anti-vascular endothelial growth factor (VEGF) agents have proven to be successful in halting the progression of wet AMD, but the frequency of treatments creates a significant burden on patients and caregivers. Moreover, no good treatments exist for dry AMD. These gene therapy clinical trials for AMD seek to target anti-angiogenic and anti-inflammatory therapeutic proteins, including vascular endothelial growth factor (VEGF) inhibitors and complement regulatory proteins. Unfortunately, no clinical trials for AMD have yet progressed to phase 3, as all previous trials ended early without evidence of clear efficacy.

Challenges to Gene Therapy

While this certainly an exciting area of research, several challenges exist to making gene therapy mainstream for a variety of ocular diseases.

First, there is currently a significant economical barrier to providing widespread gene therapy. Luxturna costs $425,000 per eye, which was shown to be justified given a lifetime of benefit to its young recipients who would otherwise suffer progressive, debilitating blindness.[9] However, this raises the question of using costly gene therapy to treat conditions that affect predominantly elderly patients, such as AMD.

Then, there are technical limitations with the existing technology. For example, the size of the desired genetic material able to be transferred with gene therapy is limited by the capacity of vectors. More complex retinal diseases will require modification of multiple genes, which may require larger or multiple vector products. Moreover, many of these genetic defects remain to be identified. Finally, the highly technical maneuvers required to deliver the gene therapy need to be safe, reproducible and consistent.

Conclusions

Gene therapy offers a promising new frontier of treatment possibilities for multiple retinal diseases, especially inherited retinal disorders. Clinical trials are also actively underway to treat more common conditions, such as AMD, though much work remains before these therapies become widely available to patients.

REFERENCES

1. Bennett J. Taking stock of retinal gene therapy: looking back and moving forward. Mol Ther. 2017;25(5):1076-1094.
2. Sheridan C (February 2011). "Gene therapy finds its niche". Nature Biotechnology. 29 (2): 121–128. doi:10.1038/nbt.1769. PMID 21301435.
3. Gene Therapy Clinical Trials Worldwide Database. The Journal of Gene Medicine. Wiley (June 2016)
4. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet. 2017;390(10097):849-860.
5. Maguire AM, Russell S, Wellman JA, et al. Efficacy, safety, and durability of voretigene neparvovec-rzyl in RPE65 mutation-associated inherited retinal dystrophy: results of phase 1 and 3 trials. Ophthalmology. 2019;126(9):1273-1285.
6. FDA Approves Spark Therapeutics' LUXTURNA (voretigene neparvovec-rzyl), a one-time gene therapy for patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy [press release]. Spark Therapeutics. December 19,2017. ir.sparktx.com/news-releases/news-release-details/fda-approves-spark-therapeutics-luxturnatm-voretigene-neparvovec . Accessed October 8, 2019.
7. Orphan Drug Act. In:1983:21 U.S.C., Ch. 29, Subch. V, Part B, §§ 360aa-360ff361, as amended.

8. Khan A, Ho A. The Eye as a Biofactory. The next phase of gene therapy. Retinal Physician, Volume: 17, Issue: Special Edition 2020, page(s): 22-25
9. FDA approves novel gene therapy to treat patients with a rare form of inherited vision loss.