The Use Of Gene Therapy In Pharmaceutical Interventions

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Abstract

There has been a renewed interest and increased investment in the field of gene therapy, primarily due to advancements in viral vector technology. This has led to the recent approval by the United States Food and Drug Administration of the first gene therapy product that specifically targets an illness caused by mutations in a single gene. LUXTURNA™ is a therapy that introduces a functional version of the RPE65 gene into retinal cells. It is used to treat retinal dystrophy associated with biallelic RPE65 mutation, a condition that causes blindness. Due to a better knowledge of the genetic causes of ocular disorders and the specific characteristics of the ocular area that make it suitable for local gene therapy, several new gene therapy initiatives are being developed. These programs aim to address hereditary retinal diseases as well as other ocular diseases. In this review, we examine the increasing amount of literature that discusses the creation and advancement of ocular gene therapy products. We focus specifically on the selection of targets and vectors, as well as the chemistry, production, and regulations involved in their development.

Keywords: Food and Drug Administration, gene therapy, pharmacy, pharmaceutical interventions.

1. Introduction

Gene therapy is the use of genetic techniques to cure human illnesses. This might include either introducing a healthy version of a faulty gene or correcting a gene to restore its normal function. The notion was first suggested in the early 1990s (1), when advancements in our understanding of human genes and the molecular underpinnings of illness development made it possible to consider genetic intervention as a means of achieving therapeutic results. During the initial phase of gene therapy, replication-defective retroviruses were employed as carriers for delivering genes.

However, optimism was quickly diminished due to concerns regarding adverse reactions, such as inflammatory

responses to the carriers and the possibility of genotoxicity resulting from the integration of the carriers into the genome. Despite the initial difficulties encountered in the early experiments, progress in the fields of virology, immunology, and other associated disciplines persisted, leading to the development of enhanced vectors that showed potential in overcoming these technological challenges. During the 2000s, adeno-associated virus (AAV) vectors, which are derived from a non-pathogenic and non-enveloped replication-defective parvovirus, became a reliable and effective method for delivering genes. This development led to a renewed interest in therapeutic applications.

In 2008, three separate groups reported the first success of clinical gene therapy. They established the safety of injecting a particular protein called retinal pigment epithelium–specific 65-kDa protein (RPE65)–expressing AAV vector into the subretinal area. This resulted in improved vision for individuals with hereditary blindness (2,3,4). Consequently, a resurgence in gene therapy was announced (5). In 2017, LUXTURNA™ (voretigene neparvovec-rzyl) developed by Spark Therapeutics, Inc. in Philadelphia, PA, was granted approval by the United States Food and Drug Administration (FDA) as the first gene therapy medication for the eye.

2. Gene Therapy

Gene therapy is being actively explored as a potential treatment for several genetic illnesses, with a special focus on ocular ailments. Specific genes associated with various eye diseases, such as hereditary blindness, have been discovered. Retinal cells in the posterior part of the eye are post-mitotic, meaning they do not divide. This characteristic enables persistent gene expression without the need of integrating the transgene into the genome. The eye has a well-defined structure with a confined and sealed physical area, which provides distinct benefits for localized administration.

These advantages include the capacity to directly see and reach the tissue. Furthermore, the blood-ocular barrier plays a role in maintaining ocular immune privilege and restricts immunological reactions to gene therapy products. Gene therapy has the potential to provide long-term treatment, reducing the burden of treatment that is typically involved with delivering medication to the back part of the eye (such as injecting anti-vascular endothelial growth factor [VEGF] biologics into the eye). The advancements in ophthalmology research, such as the development of animal models, are playing a crucial role in the increased efforts in ocular gene therapy.

Leber congenital amaurosis type 2 (LCA2), a form of inherited retinopathy caused by mutations in the RPE65 gene,

has attracted early clinical attention and achieved notable results. Clinical investigations conducted up till 2013 have shown that a single subretinal injection of AAV vectors expressing RPE65 in LCA2 patients may lead to long-lasting visual improvement and increased safety for up to 3 years (6,7,8,9,10). Bennett et al. (11) provided further evidence of the safety, immunological tolerance, and effectiveness of a second injection in the opposite eye.

A long-term assessment of patients from two previous trials (ClinicalTrials.gov NCT00481546 and NCT00643747) showed a gradual decrease in the positive effects of the treatment over time. This decline was observed in terms of retinal sensitivity, visual acuity, and functional improvement, following an initial peak that occurred 6-12 months after the treatment. This outcome highlights the difficulty of using gene therapy as a sustained therapeutic approach. The FDA granted approval for the sequential and bilateral injection of voretigene neparvovec-rzyl to treat visually impaired patients with an RPE65 mutation. This decision was based on data from a one-year randomized controlled phase III clinical study, which showed a significant improvement in vision as a result of the treatment (14).

Following the successful treatment of LCA2 patients, vector-based gene transfer is currently being investigated in clinical trials for other types of hereditary retinal diseases, such as choroideremia, Leber hereditary optic neuropathy (LHON), Stargardt disease, X-linked retinoschisis (XLRS), and X-linked retinitis pigmentosa (XLRP) (Table I). The clinical trials for LHON can be found on ClinicalTrials.gov with the identifiers NCT01267422 and NCT02161380, while the trial for Stargardt disease can be found with the identifier NCT01367444. Besides monogenic hereditary retinal problems, gene therapy is also being investigated as a potential treatment for other corneal diseases linked to inherited abnormalities. Currently, these efforts are mostly restricted to animal research (16).

Gene therapy is also an effective method for treating chronic illnesses including age-related macular degeneration (AMD) and diabetic retinopathy that are not inherited. Specifically, in the case of AMD, the current anti-VEGF treatments such as Lucentis® (ranibizumab; Genentech, Inc., South San Francisco, CA) and Eylea® (aflibercept; Regeneron Pharmaceuticals, Inc., Tarrytown, NY) necessitate regular injections into the eye, which pose considerable challenges for patients in terms of adherence and effectiveness of the therapy. There are now ongoing intensive attempts to extend the time between treatments, however the results achieved using traditional sustained-release formulations or devices has been limited up to this point. Gene therapy technologies have progressed, leading to ongoing efforts in both preclinical and

clinical stages to achieve continuous expression of a VEGF-neutralizing protein in the back part of the eye. This holds the potential for a long-lasting therapeutic effect from a single injection, as shown in Table I.

As the use of gene therapy to treat various eye diseases expands, the development of pharmaceutical goods for gene therapy is also advancing quickly, aiming to create a potent new category of therapeutic drugs. AAVs have become the primary vehicles for delivering desired genes to particular tissues, offering enhanced precision, effectiveness, and safety. The development of these intricate products, including both viral DNA and many structural proteins, encounters several technological obstacles. The formulation and manufacture of AAV products need meticulous selection of conditions to guarantee optimal stability and yield. This is because some commonly used processing processes for biologics, such as filtration or lyophilization, might result in aggregation or reduction of AAV titer. Ensuring stability is a significant hurdle in selecting storage conditions. Due to the intrinsic complexity of AAV products, a variety of sophisticated analytical methods are typically necessary to get a thorough knowledge of the physiochemical characteristics, purity, and potency of the drug ingredient (DS) and product (DP). Simultaneously, regulatory rules are being modified to provide clear regulatory standards for gene therapy products, in conjunction with these scientific improvements.

In summary, gene therapy has become a revolutionary method that offers fresh therapeutic possibilities for many eye conditions, and its full potential is only starting to be understood. Gene therapy is now in its early stages of development as a novel treatment approach, despite its rapid evolution. Our intention is to conduct a comprehensive analysis of the present state of technology, focusing on both product design and pharmaceutical development. This review does not include gene editing tools, such as those that use CRISPR-Cas9 or transcription activator-like effector nucleases (TALENs).

3. Methods of Delivery

The efficacy of gene therapy relies on the effective transportation of the genetic material to the specific cells being targeted. The majority of gene abnormalities linked to inherited retinal diseases (IRDs) primarily impacts the growth or operation of photoreceptor cells, and to a lesser degree, the cells of the retinal pigment epithelium (RPE). Transporting genes to various kinds of retinal cells poses a difficulty due to the presence of physical obstacles that must be overcome, regardless of the method of delivery. Ocular gene delivery may be achieved by many methods, such as topical application,

periocular administration, intracameral injection, intravitreal injection, subretinal injection, or suprachoroidal injection.

The route of administration is determined by the target cell type and the delivery method in practical applications. Topical instillation, which is the least invasive method of administration, is also the least effective for delivering non-viral substances. This is because the amount of nucleic acids that can actually be used by the body is usually very low, and they do not penetrate the cornea and conjunctival epithelium efficiently. As a result, this approach is not very useful for treating diseases in the front part of the eye (31).

Periocular methods, such as retrobulbar, subtenon, or subconjunctival injections, are not particularly useful for delivering non-viral substances because big nucleic acids with high molecular weight cannot easily enter ocular tissue (31). Nevertheless, viral delivery overcomes these restrictions, as shown by the effectiveness of subconjunctival injections of AAV expressing antiangiogenics in preventing corneal neovascularization in animal models (32,33). The intracameral space provides a possible pathway to deliver nucleic acids to anterior segment tissues, however it is not very effective (31). On the other hand, the introduction of adenovirus into the intracameral space has been effectively used to transport a metalloproteinase gene into cells of the trabecular meshwork for the purpose of regulating intraocular pressure in an animal model (34).

The delivery of viral-based gene therapy for retinal illnesses is often done using intravitreal and subretinal injections. The intravitreal injection is a recognized and comparatively secure method of administering medication. The preferred approach for specifically targeting retinal ganglion cells in the therapy of illnesses such as LHON, although it may also be used to target photoreceptors and the retinal pigment epithelium (RPE). Despite the seemingly straightforward route to the retina, intravitreal delivery is hindered by anatomical barriers that impede the spread of viruses into the retina, with the inner limiting membrane being the most significant obstacle (35). Methods to improve the transfer of AAV once it is administered into the eye via the vitreous humor include using enzymes to break down the inner limiting membrane or surgically removing it. In addition, there are ongoing efforts to develop second-generation AAV vectors that may overcome these anatomical limitations. For further information on AAV vectors, please refer to the discussion below.

Compared to the delivery of AAV via the vitreous humor, injecting AAV directly into the subretinal space is a more intrusive procedure that has a higher risk of causing harm to

the retinal tissue. Possible problems arising from the operation including macular holes, subretinal hemorrhage, subretinal fibrosis, and retinal detachment. Nevertheless, subretinal administration offers the most immediate and direct pathway to reach photoreceptors and the retinal pigment epithelium (RPE). While the virus's dissemination is restricted to the bleb created at the injection site, this limitation is generally considered acceptable, especially when the bleb is located in the macular region. This is because preserving or restoring visual function is of utmost importance in this area. The first ocular gene therapy to be authorized is voretigene neparvovec-rzyl, which is injected subretinally for the treatment of LCA2 (14).

Suprachoroidal administration refers to the introduction of a substance into the area between the sclera (the white outer layer of the eye) and the choroid (the layer of blood vessels below the retina). This method provides a less invasive option compared to subretinal injections. While there is minimal experience with this method of administration, a report from an animal research has shown the potential for both safety and effectiveness of delivering AAV to the retina via the suprachoroidal route (38).

4. Summary

The approval of voretigene neparvovec-rzyl in 2017 was a significant milestone in the development of gene therapy as a treatment for ophthalmic illnesses. It is expected that there would be ongoing investment in gene-based therapeutics for both inherited retinal disorders (IRDs) and other eye illnesses. The pipeline for clinical and preclinical programs is strong, with several projects now being developed. While AAV is now recognized as the dominant vector technology, lentivirus is still used and may be a viable option for delivering bigger transgenes. Ongoing research is being conducted on non-viral delivery methods, including the evaluation of the EyeCET electroporation technology in a phase I/II trial. Further refinement of AAV through directed evolution and rational design is expected to result in the development of novel vectors that possess enhanced characteristics.

These vectors are anticipated to exhibit improved efficiency in delivering genetic material to specific tissues, using less invasive methods of administration such as intravitreal or suprachoroidal injection. The development, creation, and analysis of AAV-based products often use existing expertise and techniques that have previously been developed for biologics. The regulatory expectations in the United States and European Union seem to be comparable. However, we predict that the methods and techniques used for formulating and analyzing AAV will continue to improve as its use becomes

more widespread. Additionally, we anticipate that regulatory requirements will become clearer as more AAV products are developed and approved. Gene therapy has become a formidable method due to recent scientific progress and successful clinical outcomes. It has the ability to provide enduring therapeutic advantages to tackle medical demands that have not been satisfied. We anticipate ongoing progress in the translation of gene therapy into a significant method for treating eye problems.

References

1. Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. Science. 2018;359(6372):eaan4672.

2. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008;358(21):2231–9.

3. Hauswirth WW, Aleman TS, Kaushal S, Cideciyan AV, Schwartz SB, Wang L, et al. Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. Hum Gene Ther. 2008;19(10):979–90.

4. Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008;358(21):2240–8.

5. Naldini L. Medicine. A comeback for gene therapy. Science. 2009;326(5954):805–6.

6. Boye SE, Boye SL, Lewin AS, Hauswirth WW. A comprehensive review of retinal gene therapy. Mol Ther. 2013;21(3):509–19.

7. Cideciyan AV, Hauswirth WW, Aleman TS, Kaushal S, Schwartz SB, Boye SL, et al. Human RPE65 gene therapy for Leber congenital amaurosis: persistence of early visual improvements and safety at 1 year. Hum Gene Ther. 2009;20(9):999–1004.

8. Jacobson SG, Cideciyan AV, Ratnakaram R, Heon E, Schwartz SB, Roman AJ, et al. Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. Arch Ophthalmol. 2012;130(1):9–24.

9. Simonelli F, Maguire AM, Testa F, Pierce EA, Mingozzi F, Bennicelli JL, et al. Gene therapy for Leber's congenital amaurosis is safe and effective through 1.5 years after vector administration. Mol Ther. 2010;18(3):643–50.

10. Testa F, Maguire AM, Rossi S, Pierce EA, Melillo P, Marshall K, et al. Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital Amaurosis type 2. Ophthalmology. 2013;120(6):1283–91.

11. Bennett J, Ashtari M, Wellman J, Marshall KA, Cyckowski LL, Chung DC, et al. Maguire AM. AAV2 gene therapy readministration in three adults with congenital blindness. Sci Transl Med. 2012;4(120):120ra115.

12. Bainbridge JW, Mehat MS, Sundaram V, Robbie SJ, Barker SE, Ripamonti C, et al. Long-term effect of gene therapy on Leber's congenital amaurosis. N Engl J Med. 2015;372(20):1887–97.

13. Jacobson SG, Cideciyan AV, Roman AJ, Sumaroka A, Schwartz SB, Heon E, et al. Improvement and decline in vision with gene therapy in childhood blindness. N Engl J Med. 2015;372(20):1920–6.

14. Russell S, Bennett J, Wellman JA, Chung DC, Yu ZF, Tillman A, 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):849–60.

15. MacLaren RE, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Seymour L, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet. 2014;383(9923):1129–37.

16. Solinís MÁ, del Pozo-Rodríguez A, Apaolaza PS, Rodríguez-Gascón A. Treatment of ocular disorders by gene therapy. Eur J Pharm Biopharm. 2015;95(Pt B):331–42.

17. Farrar GJ, Carrigan M, Dockery A, Millington-Ward S, Palfi A, Chadderton N, et al. Toward an elucidation of the molecular genetics of inherited retinal degenerations. Hum Mol Genet. 2017;26(R1):R2–R11.

18. Black JR, Clark SJ. Age-related macular degeneration: genome-wide association studies to translation. Genet Med. 2016;18(4):283–9.

19. Khan AO. Genetics of primary glaucoma. Curr Opin Ophthalmol. 2011;22(5):347–55.

20. Eghrari AO, Riazuddin SA, Gottsch JD. Fuchs corneal dystrophy. Prog Mol Biol Transl Sci. 2015;134:79–97.

21. Daiger SP, Sullivan LS, Bowne SJ. Genes and mutations causing retinitis pigmentosa. Clin Genet. 2013;84(2):132–41.

22. RetNet™ Retinal Information Network. Available from: https://sph.uth.edu/retnet/. Accessed 20 Nov 2018.

23. Veleri S, Lazar CH, Chang B, Sieving PA, Banin E, Swaroop A. Biology and therapy of inherited retinal degenerative disease: insights from mouse models. Dis Model Mech. 2015;8(2):109–29.

24. Stone EM, Andorf JL, Whitmore SS, DeLuca AP, Giacalone JC, Streb LM, et al. Clinically focused molecular investigation of 1000 consecutive families with inherited retinal disease. Ophthalmology. 2017;124(9):1314–31.

25. Ferrari S, Di Iorio E, Barbaro V, Ponzin D, Sorrentino FS, Parmeggiani F. Retinitis pigmentosa: genes and disease mechanisms. Curr Genomics. 2011;12(4):238–49.

26. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet. 2016;48(2):134–43.

27. Schramm EC, Clark SJ, Triebwasser MP, Raychaudhuri S, Seddon JM, Atkinson JP. Genetic variants in the complement system predisposing to age-related macular degeneration: a review. Mol Immunol. 2014;61(2):118–25.

28. Bhisitkul RB. Vascular endothelial growth factor biology: clinical implications for ocular treatments. Br J Ophthalmol. 2006;90(12):1542–7.

29. Constable IJ, Lai CM, Magno AL, French MA, Barone SB, Schwartz SD, et al. Gene therapy in neovascular age-related macular degeneration: three-year follow-up of a phase 1 randomized dose escalation trial. Am J Ophthalmol. 2017;177:150–8.

30. Heier JS, Kherani S, Desai S, Dugel P, Kaushal S, Cheng SH, et al. Intravitreous injection of AAV2-sFLT01 in patients with advanced neovascular age-related macular degeneration: a phase 1, open-label trial. Lancet. 2017;390(10089):50–61.

31. Bloquel C, Bourges JL, Touchard E, Berdugo M, BenEzra D, Behar-Cohen F. Non-viral ocular gene therapy: potential ocular therapeutic avenues. Adv Drug Deliv Rev. 2006;58(11):1224–42.

32. Cheng HC, Yeh SI, Tsao YP, Kuo PC. Subconjunctival injection of recombinant AAV-angiostatin ameliorates alkali burn induced corneal angiogenesis. Mol Vis. 2007;13:2344–52.

33. Yoon KC, Bae JA, Park HJ, Im SK, Oh HJ, Lin XH, et al. Subconjunctival gene delivery of the transcription factor GA-binding protein delays corneal neovascularization in a mouse model. Gene Ther. 2009;16(8):973–81.

34. Gerometta R, Spiga MG, Borras T, Candia OA. Treatment of sheep steroid-induced ocular hypertension with a glucocorticoid-inducible MMP1 gene therapy virus. Invest Ophthalmol Vis Sci. 2010;51(6):3042–8.

35. Dalkara D, Kolstad KD, Caporale N, Visel M, Klimczak RR, Schaffer DV, et al. Inner limiting membrane barriers to AAV-mediated retinal transduction from the vitreous. Mol Ther. 2009;17(12):2096–102.

36. Takahashi K, Igarashi T, Miyake K, Kobayashi M, Yaguchi C, Iijima O, et al. Improved intravitreal AAV-mediated inner retinal gene transduction after surgical internal limiting membrane peeling in cynomolgus monkeys. Mol Ther. 2017;25(1):296–302.

37. Bruewer AR, Mowat FM, Bartoe JT, Boye SL, Hauswirth WW, Petersen-Jones SM. Evaluation of lateral spread of transgene expression following subretinal AAV-mediated gene delivery in dogs. PLoS One. 2013;8(4):e60218.

38. Peden MC, Min J, Meyers C, Lukowski Z, Li Q, Boye SL, et al. Ab-externo AAV-mediated gene delivery to the suprachoroidal space using a 250 micron flexible microcatheter. PLoS One. 2011;6(2):e17140.