International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

Vol. 40 No. 2 September 2023, pp. 193-225

Ocular Gene Therapy

Literature Review

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Abstract – This literature offers complete information about the advances in gene treatment in the of the eye, inclusive of cornea, conjunctiva, lacrimal gland, and trabecular meshwork. We talked about gene transport systems, collectively with viral and non-viral vectors as exact as gene modifying techniques, generally CRISPR-Cas9, and epigenetic treatments, consisting of antisense and siRNA therapeutics. We moreover furnish a specific assessment of gene treatment has been examined with corresponding outcomes. Disease stipulations embody corneal and conjunctival fibrosis and scarring, corneal epithelial wound healing, corneal graft survival, corneal neovascularization, genetic corneal dystrophies, herpetic keratitis, glaucoma, dry eye disease, and specific ocular surface diseases. Although most of the analyzed consequences on the use and validity of gene treatment at the ocular surface have been offered in vitro or the utilization of animal models, we moreover talked about the on hand human studies. Gene treatment methods are nowadays viewed very promising as rising future remedies of a variety of diseases, and this area is unexpectedly expanding.

Keywords – Gene Therapy; Cornea; Corneal Wound Healing; Corneal Dystrophy; Keratitis; Corneal Neovascularization; Glaucoma; Dry Eye; Graft Survival; Non-Viral Vector; Nano-Construct; Drug Delivery; Adenovirus; Antisense; Adeno-Associated Virus; Retrovirus; Lentivirus; Sirna; CRISPR-Cas9, Retinal Treatment.

I. INTRODUCTION

Gene therapy is the approach of transfer of genetic material to remove, replace, repair, or introduce a gene in order to treat diseases [1]. Although this idea has existed for almost 50 years, gene remedy has received momentum solely lately as a promising therapy choice for many human diseases [2, 3]. After encountering various hurdles, along with serious aspect outcomes and safety and effectivity troubles in early medical studies, nowadays extra than 2,500 medical researches are underway for an extensive range of diseases, with six gene treatments already permitted by means of the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) [4].

The anterior section of the eye, especially the cornea, is a beautiful goal for gene remedy due to its accessibility and immune privilege. Current pharmacological processes for corneal diseases solely supply a momentary benefit, regularly require repeated treatment, and have at instances proved to be ineffective, requiring similarly surgical intervention. The traditional strategy additionally does no longer generally goal the root purpose of the disease. Therefore, corneal and different ocular surface issues can also considerably advantage from gene remedy processes [5–9]. In this literature, we existing a wide overview of gene remedy vectors and talked about what is acknowledged about its anterior section functions for disease therapy.

1. GENE DELIVERY SYSTEMS

1.1. Viral Vectors

Viral vectors have proven excellent promise for transgene delivery to target cells in the anterior segment of the eye [6, 7, 9]. Viral vectors are oftentimes used as vehicles for therapeutic genes due to the excessive efficacy of transduction. Even though the usage

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of viral vectors has superb results, there are additionally many obstacles and contingencies. An extensive problem is the pre-existing immunity in opposition to viral vehicles (e.g., for adenovirus) that may additionally end result in low rates of transduction and limit the expression of the therapeutic gene inside cells. In addition, viral capsids and remnant viral proteins have the capability to cause infection in their target [10].

1.1.1. Adenovirus

Adenovirus (Ad) is a double-stranded DNA virus with greater than 50 serotypes. This type of viruses has a genomic size of about 20–40 thousand base pairs [11]. Ad has turn out to be an efficient vehicle for ocular gene remedy for the reason that it is utilized to deal with short-term adjustments in gene expression [12]. Delivery of genes coding for interleukins and different cytokines, sFLT-1, c-Met, and different therapeutic genes by way of Ad has furnished cure of a number of conditions such as corneal neovascularization, limbal graft rejection, and wound healing in specific human and animal models [13–15]. Unfortunately, Ad vectors can also nonetheless keep the capability to set off adaptive and innate immunity producing adverse outcomes such as cytotoxic T-cell response [16]. Modern generations of Ad have been modified through excising their complete genome and maintaining solely inverted terminal repeats, and packaging genes with the hopes of reducing immune response and making room to raise large therapeutic material [9, 17]. The trouble that arises from mass deletion of the viral genome is that infected cells will as soon as once more have massive immune responses due to innate immune receptors that will pick out viruses and set off an immune response primarily based on conserved molecular motifs [10]. Nonetheless, recombinant Ad (rAd) vectors are being efficaciously used as vehicles for ocular gene therapy.

1.1.2. Adeno-associated Virus

Adenoviruses and adeno-associated viruses (AAV) are by far the most used viral vehicles for gene remedy. Unlike Ad, AAV vectors induce little or no immune response [16]. However, due to its prevalence in the environment, up to 90% of the populace is naturally exposed to AAV, ensuing in pre-existing immunity from neutralizing antibodies that can reduce transduction efficiency. Anti-AAV antibodies in the sera generate a various range of seropositivity and immune response to unique AAV serotypes, as unique in numerous reviews [18–21]. AAV vectors can induce both dividing and non-dividing cells. They do now not combine into the host cell genome however exist in the cells as episomal DNA, due to which they are diluted over time as the cell undergoes division [22]. In the eye, AAV serotype five (AAV5) is frequently used as a vehicle due to the fact it is recognized to supply therapeutic genes that inhibit ocular disease, like corneal vascularization, whilst solely producing an insignificant variety of adverse results [23]. AAV serotypes 6, 8, and 9 have been correctly transduced into each mouse and human corneal stromal cells. Compared to every other, AAV9 confirmed (1.1–1.4) fold greater effectivity in contrast to AAV8 and was once (3.5–5.5) fold extra positive as a delivery system compared to AAV6 [24]. Regarding the anterior segment of the eye, rAd and rAAV each have been mentioned to transduce human and rabbit corneas effectively. rAAV serotypes have been significantly and statistically higher viral vectors in contrast to Ad, though the stage of gene expression judged by means of GFP stages was once markedly greater with Ad [25]. All the rAAV serotypes examined produced wonderful however exclusive GFP staining in the corneal epithelium upon topical administration. Transduction of rAAV1 and rAAV8 produced greater staining intensities as in contrast to rAAV2, rAAV5 and rAAV7 [25]. The sub-conjunctival injection additionally produced serotype-dependent patterns, with AAV6 constrained to the corneal endothelium, whereas AAV8 successfully transduced the stroma [26].

Recombinant AAV can supply a huge range of genetic material to manipulate protein expression inside cells and alleviate some ocular diseases. Currently, two AAV-based gene treatment options have been authorized with the aid of the FDA for human use, and many are in scientific trials [27]. As corneal angiogenesis is one of the most frequent types of ocular diseases, substantial work is committed to disrupting the approaches associated to VEGF-A and PIGF1 expression by means of AAV vehicles [28]. Using AAV as a vector will doubtlessly alleviate the problem of repeated intravenous injections to result in a systemic blockade of VEGF-A that is commonly expressed in the human retina [29]. Anti-angiogenic microRNAs (miR) have additionally been utilized by means of rAAV multi-target organic remedy to decrease the quantity of corneal neovascularization [30]. In order to forestall extensive immunogenicity of the viral carrier, delivery of human leukocyte antigen G (HLA-G) was once used due to its natural induction of immune tolerance mechanisms, which would make certain decreased immune infiltration. As a result, T-cell infiltration was once decreased in alkali-burned rabbit corneas, with diminished corneal neovascularization and fibrosis [31]. A frequent trouble associated to corneal transplantation and eye disease is corneal scarring [32, 33], which should be efficiently extended the use of rAAV5 as a provider for the anti-fibrotic Smad7 gene [34]. Furthermore, transforming growth factor-β1

(TGF-β1) gene delivered through rAAV has been used in rat models of high-risk penetrating keratoplasty (PKP), with prolongation of corneal allograft survival [35].

The most recent application of rAAV vectors is through artificial contact lenses made of hydrogels of hydroxyethyl methacrylate (HEMA) with aminopropyl methacrylamide (APMA), which grant managed launch of vectors containing therapeutic genes [36]. The performance of the hydrogel was once assessed with the aid of transgene transport of reporter β-galactosidase gene recreation and crimson fluorescent protein to human mesenchymal stem cells with no obvious toxicity. Hydrogels carrying rAAV with the best useful group (H2: eighty mM APMA) yielded the most transgene expression inside the first hour and maintained transduction for a number of days. Contact lenses meting out AAV-loaded hydrogels onto the corneal surface had been located to be useful and symbolize a novel plausible technique of non-invasive ocular gene therapy.

1.1.3. Retrovirus and Lentivirus

Retroviruses have been used appreciably in gene remedy due to their fairly excessive effectivity and long-term transgene expression. They typically have an 80–130 nm capsid and a single-stranded RNA genome of 3–9 kb in size. A necessary attribute of a retrovirus is its integration into the host genome with an opportunity of insertional mutagenesis and unfold via cell division [12]. Lentiviruses share the identical characteristics as retroviruses, except that lentiviruses do no longer typically purpose insertional mutagenesis and can infect non-dividing cells like Ad or AAV [9]. Integration of retroviruses and lentiviruses into the host cell genome upon infection permits for steady gene and protein expression, making them versatile motors for gene delivery [37, 38].

Clinically pertinent equine infectious anemia virus (EIAV)-derived lentivirus has proven success in transgene transport of endostatin and angiostatin genes to inhibit corneal neovascularization [39]. Delivery of interleukin-10 gene by using lentiviral vectors extensively extended survival of corneal grafts [15]. Furthermore, subepithelial fibrosis in rat corneal stroma used to be substantially decreased by using Smad7 gene expression. Lentiviral shipping of therapeutic genes mediated the discount of TGFβ/Smad signaling brought about through diminished phosphorylation of Smad2, and Smad7 moreover down regulated the expression of pro-fibrotic TGF-β2 [40].

Integrating lentiviruses have 7% transduction efficacy after three days of application and 14.1% after 5 days in mouse and rabbit corneal epithelial cells. This makes them a less beautiful care for human use. In addition, the efficacy of transduction is dose-dependent primarily based on the quantity of lentiviral particles, with doses 5 × 103 cfu/mL and 104 cfu/mL having the easiest efficacy [41].

Most viral vectors come across the difficulty of immune cell infiltration as a protection mechanism in opposition to foreign pathogens. Additionally, it is no longer distinguished to discover insertional mutagenesis due to the fact retroviruses and, to a lesser extent, lentiviruses are infamous for randomized integration, even if their genomes have passed through changes to forestall this impact [9,23]. For this reason, retroviral and lentiviral motors nevertheless require extra investigations and perfecting earlier than they come to be relevant to humans.

2. Non-viral Delivery Vectors and Methods

Non-viral vectors are additionally used to supply therapeutic genes to cells in the anterior section of the eye. In distinction to viral vectors, non-viral vectors have been proven to be extra biologically protected as there is much less immunogenicity and pathogenicity found [42]. Additionally, a necessary gain of non-viral vectors is their typically low cost, and they may also be comfortably manufactured. However, there might also be a decrease yield of transfection with the aid of non-viral cars [43]. Various lessons of non-viral vectors that have been used in gene remedy for the anterior segment of the eye are mentioned below.

2.1. Naked DNA

Naked DNA is used besides proteins, lipids, or different buildings that would defend it. Naked DNA itself may also be utilized except a vector or automobile for gene therapy, however its structural instability can also forestall its applicable uptake by using the cells. For this reason, bare or plasmid DNA expression is up to 50% extra nice when encapsulated into an automobile or into a vector, in which gene transport into the cells will be most productive [44]. Plasmid DNA, due to its bacterial origin, carries unmethylated CpG dinucleotides and can elicit an innate immune response with the aid of activating TLR receptors [16]. This problem can be resolved through suppressing the inflammatory response via methylating or putting off the unmethylated CpG

sequences [45]. Several procedures to do so have been examined and encompass site-directed mutagenesis, elimination of non-essential areas inside the plasmid backbone, technology of artificial fragments except CpG sequences, or use of precise inhibitors of the CpG signaling pathway [46–48].Although making use of therapeutic DNA by using recombinant viral vectors would yield greater protein expression tiers inside cells, the use of non-viral cars may also have the gain of the absence of immunogenicity.

2.2. Nanoparticles and Nanopolymers

2.2.1. Metal

Introducing nucleic acids or plasmid DNA to deal with corneal illnesses by way of coating metallic nanoparticles (NP) is a dependable non-viral gene remedy method. Heavy metals and heavy metallic compounds such as silver, gold, cerium dioxide, and titanium dioxide have proven no toxicity to rabbit corneal cells. Zinc oxide as a nano service ought to stimulate the manufacturing of reactive oxidative species and over expression of apoptotic biomarkers Bax and Bcl-2 [49]. Metal NPs may also be added with pressure by using gene gun, however transfection of these gene provider particles will additionally appear when added topically, in solution, to corneal and conjunctival cells. Topical administration of poly-ethyleneimine-conjugated gold NPs containing the BMP7 gene should inhibit corneal fibrosis in vivo [50]. Gold NPs may additionally be one of the most normally used kinds for drug and gene transport as they are ultra-stable, do now not accumulate and mixture in the body, and can be made into compounds with molecular weights ranging from 800 to 6000 g/mole [51].

2.2.2. Magnetic

There are few information reporting the outcomes of magnetic nanoparticles as an approach of treatment, however it nevertheless stays a vehicle for gene therapy. Magnetic NPs are investigated due to the special bodily homes that permit them to have organic interactions with cells when they are uncovered to an exterior magnetic area [52]. Superparamagnetic iron oxide NPs (SPIONP) have been added into bovine corneal endothelial cells (CEC) and had been located to go CECs into injured areas and alter metabolic action as the concentrations of magnetic particles have been increased. There was once an enormous distinction between the metabolic undertaking of CEC (100 × 106 SPIONP/cell) with and barring a magnetic subject utilized [53]. In a latest study, alternatively of immediately handing over magnetic NPs into corneal or ocular cells, super para-magnetic NPs have been loaded into human corneal endothelial cells and injected into the anterior chamber of the rabbit cornea after Descemet's membrane stripping. This approach decreased the chance of toxicity in the eye and confirmed that magnetic cell traveling outperformed nonmagnetic cell transport via gravity in retention and efficacy [54]. By loading magnetic NPs into human CEC and guiding them with a magnetic field, drug and therapeutic gene transport have been proven to amplify up to 2.4-fold besides compromising the transfected cell viability or identification [55]. Such effects recommend that magnetic NPs might also be excellent choice candidates for gene remedy of anterior eye disease, even though greater research must be done.

2.2.3. Micellar and Liposomal Nanoparticles

Micelles are self-assembling amphipathic molecules that expose their polar surface to the exterior environment, and their non-polar parts are enclosed inside. This meeting is a feasible service of genetic cloth at its core. Micelles enable for effortless entry into tissue with little to no immune reaction. Some examples of micelles encompass naturally going on chitosan and hyaluronic acid-based polymers, in addition to poly(alkyl cyanoacrylate), poly(ε-caprolactone), and poly(butyl cyanoacrylate). The benefit of the use of micellar NPs is that their constructions are biologically like minded and biodegradable, which lets in for facilitated transfection of genetic fabric into recipient cells. The use of such NPs in the cornea stays limited. Topical utility of polymeric micelles allowed the right expression of the reporter gene with precise promotors in the epithelial and stromal corneal cubicles [56]. An aqueous combined nanomicellar components (MNF) of dexamethasone was once proven to make bigger the solubility of the drug with no cytotoxicity in remoted rabbit corneal epithelial cells [57]. Such a system should additionally be doubtlessly used to supply gene constructs to diseased corneal cells.

2.2.4. Cationic Nanoparticles

Cationic NPs are manageable gene delivering vehicles due to their biodegradable properties and stronger organic compatibility. Some biologically well matched NPs may additionally include the osmoregulatory protein albumin that is additionally a fine gene and drug transport vehicle or is lipid-coated with cationic cores. Albumin NPs loaded with bevacizumab, an antiangiogenic antibody used to deal with a multiple of cancers and unique retinal diseases, notably decreased corneal neovascularization in an animal mannequin in contrast to free bevacizumab [58]. Coating cationic NPs such as DNA-carrying chitosan with a lipid can

enlarge cellular uptake of such liponanoparticle 5-fold in contrast to a chitosan NP. The endocytosis of these cationic particles is significantly facilitated through an exterior lipid layer [59].

2.2.5. Nanopolymers

The gain of the usage of nanopolymers is that their constructions provide organic compatibility and degradability, permitting for a handy cellular uptake, as nicely as for the attachment of more than one and interchangeable useful moieties [60, 61]. As the corneal surface is uncovered to the exterior environment, it is inclined to epithelial debridement. Topically administered polymeric micelles containing anti-apoptotic genes of the bcl-2 household have been proven to decrease cell demise and heal epithelial debridement accidents [62]. Similar transport vehicles had been made to comprise β-galactosidase gene beneath the manage of corneal epithelium- and stroma-specific promotors of keratin 12 and keratocan genes, respectively. Such topically utilized micelles had been capable to result in unique gene expression in the supposed corneal components suggesting their validity for gene remedy [56].

2.2.6. Dendrimer

Dendrimers are high-quality nano vehicles for gene remedy due to their structural graph to prefer biodegradability and drug launch mechanisms. Dendrimers have three principal structural components, the central core, the relatively branched interior (that resembles dendrites) in which genetic fabric may also be intercalated, and the exterior surface with customizable purposeful groups [63]. Polyamidoamine dendrimer hydrogels designed to supply anti-glaucoma drugs proved to be extra nice than frequent PBS-formulated eye drops [64]. Dendrimer hydrogels have a notably improved uptake in human corneal epithelial cells and bovine corneal epithelium, stroma, and endothelium, by means of up to 4.6--fold vs. brimonidine and timolol maleate [64]. Dendrimer-dexamethasone gel containing the D-Cy5 gene can also additionally be injected subconjunctivally to limit corneal infection prompted through immune cell infiltration and inflammatory cytokine expression [65]. Dendrimers have been observed non--toxic in vitro in corneal and conjunctival cells submit 6-hour incubation with 0.2–20 μM nanocarriers [66]. Dendrimers have the practicable to supply the essential material to deal with an extensive vary of ocular ailments besides unfavorable sides effects.

2.3. Microinjection

Microinjections are administered via a bore glass needle that is about 0.2 μm or much less in diameter. Due to the minuscule measurement and precision of microinjections, they are extensively used as a bodily technique to supply DNA immediately into the cell nucleus or in surrounding structures that will decorate gene expression [67]. Application to cells is very correct however can emerge as tedious when more than one cells are being injected individually. Reports have proven profitable delivering of reporter genes coding for GFP, interleukin-18 that performs a function in anti-angiogenesis, Flt intraceptors to inhibit injury-induced angiogenesis, and metalloproteinase-14 to decrease corneal scarring [68–70]. Microinjections have proven incredible promise as a dependable approach of transgene transport and a viable remedy for scientific prerequisites affecting the anterior segment of the eye. Microinjections proceed to be multi-functional equipment with manageable to supply gene remedy to the anterior segment of the eye.

2.4. Electroporation

Electroporation utilizes high-intensity electric powered impulses to structure pores inside the cell membrane to facilitate plasmid gene transfer. Electro gene remedy is hard to behavior on most organs due to their inside location, however as the anterior segment of the eye is without problems accessible, it has come to be an attainable approach for treating eye diseases. Studies have proven that growing electrical depth for gene transport into the cell resulted in better gene expression [71]. The highest quality electrical area power for this approach of gene switch is 200 V/cm in view that no corneal damage, edema, or infection was once observed. Gene uptake in the cornea is multiplied via 1000-fold in contrast to injection of DNA alone, and expression will happen inside the first 6 hours after the utility [72]. The essential difficulty when the usage of electroporation at excessive intensities is that the created nearby and transient permeability will become irreversible due to the generated warmness [73]. Nonetheless, transfection of therapeutic genes, such as Kringle 5 plasminogen, into rat corneal cells by electroporation led to sturdy gene expression and fine inhibition of corneal neovascularization [74].

2.5. Sonoporation

Similar to electroporation, sonoporation physicaly creates transient and localized pores inside cell membranes by means of ultrasound waves, therefore facilitating the switch of DNA to the nucleus. As in contrast to naked DNA, sonoporation can extend the quantity of therapeutic gene expression up to 15 – fold, however electroporation nevertheless yields a greater efficacy of protein expression [75]. Both physical modalities exhibit promise in gene traveling as neither of them has been mentioned to purpose considerable immunogenicity or pathogenicity. But they are constrained as the dimension of the molecule delivered may additionally have an effect on the quantity of transfection [76]. Research in vivo on non-human tissue counseled that it is feasible to use sonoporation as a vehicle for gene therapy, however there is now not sufficient records on how it can also work in vivo on human tissue.

2.6. Iontophoresis

Iontophoresis is an approach used to supply ionized molecules via the cell membrane by using transient and localized pores that are created through low currents [77]. A direct electrical modern-day might also be utilized to the cornea at 0.5–5.0 mA/cm2, and for the conjunctiva, it may additionally be utilized at 0.5–20 mA/cm2 for 30 min. Gene or drug travelling throughout the cell membrane by iontophoresis has been said to enlarge by means of 2.3- and 2.5-fold for the cornea and 4.0- and 3.4- fold for the conjunctiva, respectively; the switch again to baseline ranges in rabbit cornea and conjunctiva as soon as the modern-day was once no longer utilized [78]. Introducing genes and capsules into the cornea utilising contemporary is carried out by using loading the electrode with, for example, riboflavin answer and connecting it to a modern generator aiming at improving corneal cross-linking in keratoconus sufferers after epithelial debridement [79]. Three extraordinary protocols have been assessed to deal with corneal thinning sickness keratoconus, one of which is iontophoresis. Compared to traditional and accelerated methods, iontophoresis has the gain of non-invasively enhancing riboflavin penetration for the duration of the cornea. However, even with profitable outcomes with iontophoresis in lowering or heading off epithelial debridement in keratoconus treatment, the traditional system nevertheless stays most effective, being quick, safe, and subtle adequate for thin corneas [80]. Nonetheless, iontophoresis may additionally turn out to be trendy system for treating anterior ocular ailment due to having a brief procedural length of 10 minutes and being greater relaxed for patients. However, long-term research nevertheless want to be accomplished to in addition determine the efficacy and dangers that come from the use of this approach for corneal cross-linking [81].

2.7. Gene Gun

A biolistic particles delivering system, additionally recognized as a gene gun (ballistic gene transfer), is an instrument that lets in for the switch of DNA, RNA, or proteins into tissues and cells through coating them with inert, heavy metal (such as gold or silver), making micro-projectiles and handing over them with force. The gain of the use of a gene gun in the corneal epithelium is that the utility yields excessive stages of therapeutic gene expression except inflicting measurable unfavorable consequences [82]. Opioid growth factor and its receptor (OGFr) negatively adjust ocular surface epithelial proliferation and wound healing. To block this bad rules in rat cornea, the switch of OGFr plasmid antisense DNA (intended to inhibit OGFr) to the injured corneas in vivo was once used with the aid of gene gun shipping and resulted in the acceleration of wound restoration [83, 84]. The outcomes of gene gun delivered gold-coated vector DNA for IL-4, and CTLA-4 genes have been additionally explored vs. the use of gene gun in the model of corneal grafting. The manner itself precipitated elevated migration of F4/80+ macrophages into the stroma, impeding graft survival. The enlarge ought to be a response to eye inflammation due to the application. However, treating recipient tissue with vector DNA the usage of a gene gun extended graft survival [85]. The statistics exhibit that this technique must be used with warning for plasmid DNA delivery, as there are elements limiting its efficacy, together with the quantity of genetic fabric supplied, the pressure used at some point of application, shallow or deep penetration, the wide variety of cells penetrated, and temperature. With higher pressure applied, there is greater harm or irritation found in the affected cells. The Gene gun approach must be similarly investigated in the cornea [86].

2.8. Laser

The traditional excimer laser is used to right eye prerequisites such as astigmatism, myopia, and hyperopia. Photorefractive keratectomy surgical procedures using this laser have the motive of reshaping the cornea, however a frequent unfavourable impact is a corneal haze [87]. More recently, the femtosecond laser used to be delivered that has the benefit of ultrafast and ultrashort pulses that want appropriate recuperation of the cornea [88]. Due to its excessive precision and safety, the femtosecond laser has been used to create minuscule stromal pockets of one hundred ten μm depth to introduce genes into ex vivo pig cells to verify the

efficacy of gene expression [89]. Once the pocket has been created, therapeutic genes might also be added at the web site by injection. As viral gene switch has been assessed with this approach [89], the subsequent steps should consist of introducing non-viral vehicles and doubtlessly attempting this technique on human cells or ex vivo human organ cultures. The femtosecond laser has additionally been used to spark off carbon NPs that elevate genetic fabric in the ex vivo human corneal endothelium [90]. As a result, macromolecules can be utilized to the human cornea with an excessive trveling rate.

2.9. Chemical

Both synthetic and natural chemicals, along with lipids and polymers, have been broadly examined as vehicles for therapeutic gene travelling in the in vivo models of ocular gene therapy. Cationic lipids 1,2-dioleoyl-3-trimethylammonium-propane and dioleoylphosphatidyl-ethanolaminehave have been used in transfection mixtures to supply genes to human epithelial cells in culture and to corneal rabbit epithelium in vivo as eye drops [91]. Furthermore, a concoction of 5 special non-viral lipid vectors used to be utilized to human epithelial cells, which resulted in up to a 17% expand in transgene transport with almost no cytotoxicity [92]. What makes these cationic lipids environment friendly as a service is their affinity to negatively charged DNA.

2.10. Antisense Oligonucleotides

The use of short single-stranded DNA that interacts with mRNA to block the translation of a precise protein is extensively used in gene therapy. These antisense oligonucleotides (AONs) are complementary to their targets and, as soon as bound, mark mRNA for degradation to forestall protein synthesis. The gain of AONs in contrast to genetic knockdowns or knockouts is that they are gene-specific and do no longer purpose adjustments in the expression of different genes [93]. There are various variations of AONs, with the Morpholino kind being preferable due to its stability, gold standard goal specificity, and resistance to nuclease attack [94]. What makes the antisense use distinctive is that it can be noninvasive, as AONs can be utilized topically thru eye drops. In the eye, a scientific segment was once first carried out in 2014 to get right of entry to the efficacy of AON (aganirsen) concentrated on insulin substrate-1 receptor expression to block corneal neovascularization and located it fine [95]. The aganirsen topical eye drops correctly inhibited corneal neovascularization in keratitis sufferers lowering the need for corneal transplantation. Similarly, GS-101 AON, a mighty anti-angiogenic compound, used to be located most gold standard when utilized at a dose of 86 μg per day, inhibiting and inflicting regress of corneal neovascularization [96].

Introducing AONs via non-viral motors may additionally render it much less immunogenic. However, it used to be suggested that AONs concentrated on TNF-α and used in opposition to herpetic keratitis may nevertheless intervene with the antiviral response and consequently lead to recurrence of herpetic stromal keratitis [97, 98]. AONs may additionally be a doable tool to right molecular genetic disorders, such as Fuchs' endothelial corneal dystrophy (FECD), via focused on the feel RNA transcript of TCF4 triplet repeat expansion, which will reverse the poisonous nuclear RNA foci formation as properly as splicing defects [99]. In addition, AONs are realistic equipment of ocular gene remedy due to the small quantity wanted for the application. They can additionally be conveniently synthesized and modified for stability, for instance, to produce Morpholino versions that have shown efficacy for corneal wound recuperation [100]. The efficacy of AONs may additionally be structured on the stage of the ocular disease. Generally, sufferers who are at the early levels of FECD would reply higher to AON gene remedy on account that they have no longer but skilled massive endothelial cell loss due to TCF4 repeat expansion-mediated toxicity [101]. The performance of AON can be maximized when used with different techniques for facilitating drug delivery, such as nanoconstructs or iontophoresis. When used together, AON switch is increased, the delivered AONs can persist for 24 hours and might also end result in decrease expression of goal genes for as many as 7 days as mentioned via Gibson et al. 2017, though they may additionally act even longer [102]. It ought to be stated that in the cornea, free AONs can effectively, even though slowly, transduce the endothelium. Still, the epithelium requires extra equipment for delivery, such as nanoconstructs or transfection enhancers [100, 103].

2.11. siRNA

Interfering RNA (siRNAs) are a classification of double-stranded, noncoding RNA molecules that are about 20–25 base pairs, whose characteristic is to alter the gene expression of mRNA via concentrated on them for degradation, eventually main to silencing of that goal gene [104]. siRNAs have been examined in a number of ocular illnesses such as retinal disorders, glaucoma, Meesmann's epithelial corneal dystrophy (MECD), wound healing, and neovascularization [104–107]. Most of these research have been performed on rabbit, mouse, and rat tissues, even though some had been examined in human diseases, and some siRNAs are in the medical trials [108]. siRNAs can be delivered as bare constructs, interior NPs, with cell-penetrating peptides or

as components of plasmids as brief hairpin RNA (shRNA) [108–112]. Although they are extensively used notably to knock down gene expression, there are some worries about their off-target effects, stability, and quick length of their motion [108]. However, latest advances in their format would make these shortcomings much less great [104].

2.3. Next Generation of Genome Editing System: CRISPR-Cas Delivery by using Viral and NonViral Vehicles

The CRISPR-Cas9 genome modifying system is a pretty novel device for gene remedy application in the anterior segment of the eye. Like most drugs and therapeutic material, the CRISPRCas9 system can't be delivered systemically due to the blood-ocular barrier [113]. Fortunately, all of the aforementioned cars for drug travelling are viable strategies to supply CRISPR-Cas9. The largest challenge with the use of these vehicles is their carrying capacity; they are frequently too small to elevate the full genome modifying system. In AAV vectors, the most ability for genetic fabric is about 5 kilobases [114], 36 kilobases in Ad vectors [115], and up to 10 kilobases in lentiviral vectors [116]; all of which encompass inverted terminal repeats. A dual-AAV plan has been used to right this problem by using turning in the Cas9 nuclease and the sgRNA cassettes in two separate viral cars yielding excessive expression of the genome enhancing machine [117]. Further engineering ought to be investigated to optimize the area inside these contemporary vehicles. Ad has been used to supply CRISPR-Cas9 therapy for myocilin (MYOC)-associated glaucoma. The enhancing elements have been located to be effectively delivered into most important human trabecular meshwork cells and in MYOC mouse fashions for fundamental open-angle glaucoma. Transduction effectivity of up to 70% the usage of Ad5-cas9 or Ad5-crMYOC was once suggested alongside a substantial discount in IOP. Reports confirmed Cas9 expression in the corneal endothelium and components of the iris and ciliary physique [118]. Although now not many research have been performed the usage of AAV, Ad, and lentivirus to supply CRISPR-Cas9 substances to the anterior phase of the eye, a few have been achieved on the posterior ocular travelling by means of viruses. By the use of AAV and lentivirus, researchers have focused retinal pigment epithelial (RPE) cells [119] and efficiently transduced the modifying device due to the large carrying capability in contrast to Ad vectors [116, 120].

CRISPR-Cas9 gene-editing gadget delivered through electroporation used to be used in mouse retinas to take a look at the function of cis-regulatory module B108, an enhancer of a transcription component Blimp1 that controls the appropriate ratio of rods and bipolar cells. The centered areas of the genome should be efficaciously deleted in vivo [121]. In a rat model of autosomal dominant retinitis pigmentosa, a subretinal injection of plasmid bearing information RNA/Cas9 mixed with electroporation was once in a position to disrupt the mutant Rho(S334) allele of the rhodopsin gene, which avoided retinal degeneration and extended imaginative and prescient [122]. Recently, CRISPRRNP was once packaged into glutathione (GSH)-cleavable covalently cross-linked polymer or a nanocapsule and delivered to the retina, displaying useful effects [123]. The benefits of the usage of this nanocapsule for transport are its biodegradability and lack of toxicity. Another NP transport of CRISPR-Cas9 by way of silica–metal–organic framework (SMOF) hybrid nanoparticle used to be mentioned to have 7% expression of the edited gene in the retinal location [124].

Genome enhancing by CRISPR-Cas9 is a rising subject matter of hobby in gene therapy. Since there are no longer many research that show the travelling of the CRISPR-Cas9 device to the anterior segment of the eye, extra lookup should be done. The successes stated from the use of viral and non-viral strategies for shipping of this gene-editing device to the posterior eye are a beginning factor for similarly investigations associated to the anterior segment pathologies.

2.4. Retinal gene therapy

The advances in AAV vector production, efficacy, and flexibility precipitated a new wave of medical exercise in the 2000s. Researchers now had the ability to introduce therapeutic plasmids with minimal difficulty for immune or inflammatory response with a degree of tissue specificity in no way viewed before. With an association perception of AAV vectors and gene remedy biology, ocular problems grew to become a goal for gene remedy trials. Although many issues are regularly polygenic in dysfunction, ocular problems are well-understood for having character genes accountable for a range of stipulations [16]. This makes ocular issues attractive because changing a single gene each minimizes the payload transport for AAV vectors whilst fending off viable mistakes that can occur with giant transgenes. Secondly, the manageable for a single dose of gene remedy shipping is an enormous enhancment over the cure burden of general intravitreal injections to deal with retinal diseases.

This makes ocular problems attractive considering the fact that changing a single gene each minimizes the payload transport for AAV vectors whilst heading off viable blunders that can occur with giant transgenes. Secondly, the conceivable for a single dose of gene remedy travelling is a full-size enchancment over the remedy burden of familiar intravitreal injections to deal with retinal

diseases. These strengths of AAV accepted its use in the earliest scientific trials of ocular gene therapy. In three separate trials started out in 2008, AAV2 vectors carrying a purposeful reproduction of retinal pigment epithelium (RPE)-specific 65-kDa protein gene (RPE65) have been injected subretinally into sufferers with RPE65-Leber congenital amaurosis. In all three trials, sizeable enchancment used to be viewed in enhancing visible dysfunction [17–19]. In doing so, the promise of gene remedy lived up to the expectations initially set through Theodore Friedmann. The effects of these three trials set the groundwork main to the FDA-approval of voretigene neparvovec-rzyl (Luxturna), the first FDA-approved ocular gene therapy.

2.5. RETINAL GENE THERAPIES ;Voretigene neporvovec-rzyl (Luxturna)

Voretigene neporvovec-rzyl from Spark Therapeutics is a one-time AAV2-based subretinal gene remedy indicated in sufferers demonstrated with biallelic RPE65 mutation-associated retinal dystrophy which manifests both as Leber congenital amaurosis two or uncommon types of retinitis pigmentosa (RP). LCA influences 1 in 30,000–81,000 human beings and is a extreme and early kind of inherited retinal disease-causing childhood blindness [125]. LCA is characterised through giant visible impairment beginning in infancy or early childhood, looking nystagmus, a slow to near-absent pupillary response, and severely subnormal to non-detectable electroretinography [125,126].Voretigene neparvovec-rzyl works by means of handing over a useful reproduction of the gene RPE65 to retinal cells by using subretinal injection. Normal RPE65 protein converts all-trans-retinol to 11-cis-retinol, permitting the formation of chromophore 11-cis-retinal for the duration of the retinoid cycle; this system is necessary to the organic conversion of mild pictures into electrical alerts transported to the talent through the optic nerve. In LCA, decreased or absent ranges of RPE65 disrupts the retinoid cycle, ensuing in imaginative and prescient impairment [127].

By transducing the RPE cells with the transgene, practical RPE65-specific kiloDalton protein is thereby produced. The approval of voretigene neparvovec-rzyl now not solely made it the first-ever ocular gene remedy to turn out to be on hand to patients, however additionally represented the fruits of a massive physique of preceding scientific work. Aside from the earliest research described above, many different early-phase research have been performed inclusive of one Phase half trial involving 12 individuals who acquired unilateral, subretinal injections of AAV2-hRPE65v2 in the worseseeing eye in a dose-escalation find out about evaluating two doses, 1 × 1010 and 1 × 1011 vector genomes [128–132]. One affected person increased visually in each the handled and untreated eyes whilst three sufferers skilled a visible decline. Six sufferers extended in dark-adapted perimetry whilst three increased in low-light navigation, and 5 sufferers extended in microperimetry. These outcomes seemed to wane past the first-year post-treatment. Additionally, no most important systemic immune-related adverse events (AE) have been noted, even though 6 of 10 sufferers with foveal-involving injections had retinal thinning and three sufferers in the higher-dose crew have been located to have intraocular inflammation. Ultimately, the encouraging consequences of the Phase half trial led to a Phase three trial concluding that voretigene neparvovec-rzyl used to be well tolerated ordinary with no product-related serious unfavourable consequences or immune responses noted. The principal effect evaluated the exchange in the overall performance of a study-specific multi-luminance mobility take a look at (MLMT) from baseline at 1 year, which used to be met as the handled crew made statistically tremendous enhancements in their give up score. Secondary endpoints evaluated full-field stimulus thresholds and Goldmann visible area testing, with sizeable enhancements for each measures in the handled crew over the manipulate team [133]. The most frequent ocular unfavorable outcomes had been surgery-related and increased intraocular stress (20%), cataracts (15%), ocular infection (10%), and retinal tears (10%). These usual favorable outcomes determined in this Phase three trial led to the approval of voretigene neparvovec-rzyl for the cure of biallelic RPE65 deficient-associated retinal disorder through the United States Food & Drug Administration in December 2017. Voretigene neparvovec-rzyl is presently being administered at ten remedy facilities in the United States.

2.6. ADVM-022

ADVM-022 with the aid of Adverum Biotechnologies is presently in a Phase 1 open-label scientific trial (OPTIC) as an in-office intravitreal gene remedy to deal with neovascular age-related macular degeneration (nAMD), the most frequent motive of imaginative and prescient impairment in men and women 50 years of age and older, and a Phase two trial (INFINITY) for the therapy of diabetic macular edema [134]. Pathologically, vascular endothelial growth factor (VEGF) promotes the improvement of choroidal neovascularization and the subsequent development of nAMD. As such, anti-VEGF cures have been the major center of attention of pharmacological intervention for persons with nAMD. Aflibercept is an antiVEGF protein which is generally used in the remedy of retinal illnesses and has been FDA-approved on the grounds that 2011. ADVM-022 ambitions to deal with nAMD via a single intravitreal injection using the AAV.7m8 capsid to supply a codon-optimized cDNA expressing an

aflibercept-like protein . With ADVM-022, the want for repeated intravitreal injections each 4–8 weeks can be mitigated with an ensuing minimize in therapy burden [135].

Nonhuman primate research with ADVM-022 have determined that a single intravitreal injection of ADVM-022 resulted in sustained expression of aflibercept for 30 months [135]. Moreover, the aflibercept was once in a position to inhibit choroidal neovascularization in a nonhuman primate mannequin of nAMD, even when injected 13months prior to lasering [136]. These findings led to the initiation of the OPTIC trial, which is the first in-human trial with ADVM-022 for sufferers with nAMD. The 2-year ongoing OPTIC Phase I trial has enrolled four cohorts of previously-treated sufferers with neovascular AMD: cohorts 1 (6 patients) and four (9 patients) obtained the excessive dose (6 × 1011 vg/eye), and cohorts two (6 patients) and three (9 patients) acquired the low dose (2 × 1011 vg/eye). These sufferers have been closely pre-treated prior to enrolling in OPTIC with a common of 9 injections in the preceding yr in cohorts 1, 2, and 3, with 7 injections in cohort four The principal effect evaluates the protection and tolerability of one injection of ADVM-022, whilst the 2d goals verify the best-corrected visible acuity (BCVA), anatomic results of spectral-domain optical coherence tomography, and the want for anti-VEGF supplemental therapy. Steroid prophylaxis of ocular irritation used to be used in all four cohorts, however with specific regimens per cohort. A 13-day route of oral steroid with tapered topical eyedrops had been used in cohorts 1 and two whilst cohorts three and four have been handled with a 6-week path of topical steroid eyedrops. The cohorts have comparable demographics and anatomical baselines with cohort three having a greater common central subfield thickness (CST) at baseline. Following a single intravitreal injection of ADVM-022, the highdose cohorts validated a 96% discount in the imply annualized frequency of anti-VEGF injections and the low-dose cohorts established an 85% discount in redress [137].

The BCVA and CST in cohort 1 remained steady besides supplemental cure at a median 104-week follow-up [137,138]. Subjects maintained −1.3 letters and −8.7 μm discount in CST for the duration of this time period. Cohort two maintained BCVA (−1.5 letters in all sufferers and −1.0 letters in supplemental-free patients) and CST (−28.2 μm in all sufferers and −30.3 μm in supplemental-free patients) over a median 84-week follow-up. In cohort 3, the BVCA (+1.4 letters in all patients and +4.3 letters in supplemental-free patients) was once maintained and the CST (−134.4 μm in all sufferers and −181.7 μm in supplemental-free patients) elevated via a median 56-week follow-up. In cohort 4, the BVCA (−0.2 letters in all sufferers and −0.4 letters in supplemental-free patients) used to be maintained and the CST (−77.1 μm in all sufferers and −77.3 μm in supplemental-free patients) increased thru a median 36-week follow-up.

No ADVM-022-related non-ocular AEs have been stated at this time. Inflammation has been the predominant AE in this trial, with ocular inflammation predominately affecting the anterior segment stated in particular in the higher-dose cohorts. No posterior inflammation, vasculitis, or endophthalmitis has been reported. A lowering fashion in irritation has been considered over time however more than one patients have required long-term topical drops, mainly in the high-dose group. The lower dose has been related with fewer instances of inflammation. All treatment-related ocular AEs were slight (80%) to reasonable (20%). An AE of one-of-a-kind hobby (AESI) was documented in the high-dose Cohort 1 of moderate recurrent uveitis unrelated to ADVM-022 treatment. This affected person was once positively responsive to steroid eyedrops. Two sufferers had slight AEs of IOP elevation that resolved with Combigan eyedrops [139]. Importantly, in those patients who consented for aqueous taps in the OPTIC trial, there used to be sustained long-term aflibercept protein expression, out to 104 weeks in one patient. With the exception of one affected person who required rescue injections, all aflibercept protein stages expressed as an end result of ADVM-022 gene therapy had been observed to be in therapeutic range when compared to the commercially on hand Eylea (aflibercept recombinant protein, Regeneron Pharmaceuticals) injection [135,137,140]. As this trial is ongoing and a long-term extension is planned, we will continue to learn greater about protection and efficacy of ADVM022. These results will guide the plan of future research to consider ADVM-022 as a practicable option for the treatment of nAMD.

Adverum Biotechnologies performed a 2d trial, INFINITY, for the use of ADVM-022 for diabetic macular edema (DME). In late April of 2021, Adverum unmasked the trial following a SUSAR of hypotony in the handled eye of a DME affected person in the high-dose cohort of the trial 16–36 weeks after administration of ADVM-022. Close monitoring and aggressive therapy led to no enchancment in the patient’s condition. Similar SAEs have now not been discovered in the low-dose cohorts. Adverum has discontinued improvement and investigation of ADVM-022 for DME at this time [141].

2.7. RGX-314

RGX-314 (REGENXBIO Inc.) makes use of an AAV8 related gene remedy for the therapy of nAMD. RGX-314 expresses a monoclonal antibody fragment comparable to ranibizumab. As a hooked up antiVEGF therapy, ranibizumab is a humanized monoclonal antibody fragment that binds to human VEGF-A to suppress choroidal neovascularization [142]. RGX-314 makes use of subretinal or suprachoroidal shipping and gives the possible for secure anti-VEGF antibody manufacturing whilst lowering the burden of more than one intravitreal injections.42 sufferers with extreme nAMD have been enrolled in the ongoing Phase I/IIa trial of RGX-314 searching at protection and efficacy of RGX-314 delivered subretinally with the aid of transvitreal approach. The remedy has been commonly well-tolerated with no reviews of unusual immune response, drug-related ocular inflammation, or post-surgical irritation made therefore a ways [143]. 20 SAEs have been said in thirteen sufferers with one maybe drug-related SAE of giant minimize in imaginative and prescient in the high-dose Cohort 5. 87% of the most frequent nonserious ocular AEs have been assessed as slight with the frequent AEs being postoperative conjunctival hemorrhage in 67% of patients, post-operative infection in 36% of patients, and eye inflammation eye pain, and postoperative visible acuity discount in 17% of sufferers every [142]. There has additionally been no drug-related ocular inflammation, strange postsurgical inflammation, or clinically decided immune responses located to date [143].

Retinal pigmentary modifications at the web site of the subretinal bleb have been seen in 67% of the patients. Thus far, the efficacy records from the trial has been promising. Patients in cohorts 3, 4, and 5 have protected 6, 12, and12 sufferers respectively at the doses of 6 × 1010 GC/eye 1.6 × 1011 GC/eye and 2.5 × 1011 GC/eye respectively. 2-year facts from cohort three has proven BCVA positive aspects of +14 letters. In cohort four and 5, at 1.5 years, imply BCVA in these two cohorts has modified +1 letters and −1 letters from baseline respectively. Central retinal thickness has modified two µm in cohort three and −46 and −93 µm in cohorts four and 5 respectively. Patients in cohort three have benefited from a minimize in imply exchange in annualized injection of 66.7% at three years whilst cohort four and cohort 5 sufferers have skilled a 58.3% and 81.2% reduction, respectively, at 1.5 years.The find out about is ongoing and sufferers will be observed in the extension trial for a complete of 5 years from enrollment. We will proceed to analyze about efficacy and protection of RGX-314 thru different research and an increasing scientific program. The pivotal ATMOSPHERE trial is assesing subretinal injections of RGX-314 for nAMD patients. The Phase two trials AAVIATE and ALTITUDE for neovascular AMD and diabetic retinopathy respectively, will check RGX-314 administered by way of the suprachoroidal route.

2.8. GT-005

Gyroscope's Therapeutics' FOCUS trial using GT-005 is a gene remedy designed to goal sufferers with dry AMD. Dry AMD debts for 85–90% of all AMD instances [144]. Unlike nAMD, there are presently no high-quality remedies reachable for dry AMD. Advanced dry AMD, additionally referred to as geographic atrophy (GA), is characterised by means of irreversible degeneration of RPE cells and their overlying retinal photoreceptors, main to everlasting imaginative and prescient loss.Though there are no mounted redress for dry AMD, one proposed pathological mechanism includes dysregulation of the complement system. It is believed that an overactive choice pathway of the complement system, an arm of the innate and adaptive immune systems, is in part accountable for the improvement of dry AMD. This speculation is supported with the aid of Gene-byEnvironment Wide Association Studies (GEWAS) and the accumulation of complement proteins in drusen which are insoluble extracellular aggregates in the retina attribute of AMD [145].

GT-005's therapeutic thought is the AAV2 vector turning in a plasmid assemble expressing ordinary Complement Factor I (CFI) protein, a herbal inhibitor of the complement device [146]. GT-005 is designed to allow cell transduction and set off secretion of CFI. It has the conceivable to permit for constitutive expression of CFI after a single administration and to keep away from the sawtooth dynamics of repeated intravitreal injections. CFI is a herbal regulator and features to maintain the complement gadget in balance. Given this fundamental role, CFI is properly ideal for gene therapy. GT-005 is presently present process assessment for security and efficacy in more than one Phase 1 (FOCUS) and Phase 2 (HORIZON and EXPLOREe) scientific trials.

At this time, no GT-005- associated SAEs have been observed. 1 viable GT-005-related AE of choroidal neovascularization of average severity has been found and dealt with with anti-VEGF therapy. 12 surgery-related AEs have been located with 75% labeled as slight and 25% labeled as moderate. Two ocular AEs have been discovered with extended IOP. One case of multiplied IOP resolved with drops. The 2nd case self-resolved. No symptoms of GT-005-related infection or immune response have been

found to date [147].The find out about is ongoing and continues to be monitored carefully to check the longer-term security and efficacy of GT-005.

2.9. HMR59

HMR59 by means of Hemera Biosciences is an AAV2 vector-based gene remedy expressing sCD59 administered intravitreally 7 days after a single intravitreal injection of anti-VEGF treatment. CD59 is located on the surface of RPE cells and is believed to inhibit the formation of the MAC complicated throughout late-stage complement device activation. CD59 prevents the formation of the MAC complicated through inhibiting the recruitment of C9 to the C5b-C8 complicated [148]. By concentrated on the MAC complicated specifically, the upstream complement cascade stays intact. Because an overactivation of the complement machine is proposed to be one of the pathological pathways main to nAMD, HMR59 objectives to upregulate CD59 expression on RPE to defend in opposition to the complement cascade suspected in inflicting macular neovascularization.

HMR59 is presently below evaluation in two separate Phase 1 scientific trials. Trial HMR-1002 (NCT03585556) is underway to examine the efficacy and security of two doses of the HMR59 for remedy towards nAMD. 22 patients will be injected with a low-dose remedy of 3.56 × 1011 vg/eye, and three sufferers will be injected with a high-dose therapy of 1.071 × 1012 vg/eye. Anti-VEGF remedies will be injected at Day zero to deal with per fashionable of care. The find out about is ongoing and will be evaluated for long-term follow-up security and efficacy [149]. HMR59 is additionally being evaluated for use towards dry AMD in the HMR-1001 (NCT03144999) trial. 17 patients will have been injected with both a low, mid, or excessive dose of HMR59 in the affected eye [150]. Thus far, HMR59 has normally been nicely tolerated. three of 17 topics developed slight vitritis that resolved with topical steroids, A 23% discount in GA has been determined at the very best dose, and no cure eyes have transformed to nAMD in the course of the 18-month follow-up. HMR-1001 is additionally ongoing and will be evaluated for long-term follow-up security and efficacy [151].

3. Routes Of Administration For Drug Delivery

There are 4 predominant routes of drug administration to deal with ailments of the anterior section of the eye, particularly topical, subconjunctival, intracameral, and systemic. The most vital elements of ophthalmic drug administration are the period of drug release, goal area and ocular barriers, and affected person compliance [152]. Based on these factors, every mode of administration has its benefits and limitations.

3.1. Topical

Topical transport is the most frequent structure of ocular drug shipping for anterior section diseases. It is simple, non-invasive, self-administrable, and normally formulated as eye drops. Several elements such as blinking, answer drainage, and the tear movie decrease the bioavailability of topically utilized drugs. Due to regular tear turnover, topical capsules get washed out of the eye and require repeated application, which is affected due to negative affected person compliance [153]. Recent research have centered on the use of nanocarriers to enhance bioavailability of topically applied drugs. Nanocarriers have a higher capacity to adhere to the ocular surface, sustained delivery, and increased shipping of poorly soluble capsules [154]. Corneal and conjunctival limitations can additionally create impediments to positive topical therapy due to the a variety of layers of the cornea and the presence of conjunctival blood capillaries [155]. It is additionally vital to be aware that whereas this route is desired for treating various corneal and conjunctival disorders, many profitable research making use of topical administration are carried out in mice that have thinner cornea (~150 μm thick) as in contrast to people (~550 μm thick). Hence, to attain the deeper layers of the cornea, different routes of drug administration, such as intrastromal transport or penetration enhancers, may also be required.

3.2. Sub-conjunctival

Sub-conjunctival implants or injections are administered beneath the conjunctiva of the eyeball (epibulbar) or the conjunctiva lining the eyelid (subpalpebral). Sub-conjunctival shipping bypasses the corneal barrier and additionally circumvents the washout and precorneal drainage barriers related with topical drug use. Injected capsules may also be designed as sustained launch formulations that grant advantageous cure for longer period or excessive awareness with a single injection [156].

3.3. Intracameral

Intracameral injections are administered without delay into the anterior chamber. This approach offers excessive awareness of the drug and circumvents the corneal aspect results viewed with topical administration. It is frequently used to supply an anesthetic or to inject antibiotics to forestall endophthalmitis that may also show up at some point of cataract surgical operation [152].

3.4. Systemic

Systemic drug travelling is performed by way of intravenous, intraperitoneal, or oral administration. It is most regularly used for treating posterior section illnesses however has additionally been used to deal with stipulations affecting the eyelids and sclera, as properly as in the therapy of herpetic stromal keratitis. However, the blood-aqueous barrier limits the wonderful transport of a drug with the aid of this approach to the anterior section [156]. Moreover, systemic transport is additionally related with aspect consequences and toxicity associated to excessive drug attention [157].

4. Applications Of Gene Therapy In Disease

Diseases of the anterior phase of the eye, mainly corneal opacity, are the fourth main reason of visible impairment and blindness, as mentioned via the World Health Organization [158, 159]. These illnesses embody a broad range of pathological conditions, along with quite a number kinds of trauma, infections, and hereditary diseases, amongst others, that reason ordinary wound healing, inflammatory reactions, corneal cloudiness, scarring, and graft rejection. Due to its relative accessibility, the anterior section is uniquely perfect for gene transport to deal with these diseases. We describe right here the purposes and advances of gene remedy for the remedy of more than a few such conditions.

4.1. Corneal and Conjunctival Fibrosis and Scarring

The wound restoration response to corneal harm regularly effects in stromal scar formation and fibrosis characterised through the emergence of myofibroblasts and the unusual deposition of extracellular matrix (ECM) proteins. TGF-β performs a key function in the transdifferentiation of stromal fibroblasts to myofibroblasts and the pathogenesis of ocular fibrosis [160]. The expression of α-smooth muscle actin (α-SMA) due to TGF-β undertaking is the hallmark of myofibroblast phenotype alongside with the deposition of more than a few ECM proteins such as collagens sorts I, III, IV, and fibronectin. The immoderate synthesis of ECM proteins alters the properties of the current ECM, ensuing in scarring and haze [161]. Therefore, TGF-β has emerge as the predominant goal of gene remedy for the prevention and remedy of fibrotic ocular floor disease.

For anti-fibrotic gene therapy, Mohan et al. [162] have considerably studied decorin, a small leucine-rich proteoglycan [163] concerned in ECM organization. Decorin varieties a complicated with TGF-β, for that reason reducing its bioavailability, blockading its binding to its receptors, and ensuing in diminished fibrotic undertaking [164–166]. Transfection of decorin gene cloned into the mammalian expression vector in human corneal fibroblast cultures resulted in a downregulation of expression of α-SMA, collagens sorts I, III, IV, and fibronectin that used to be brought on via TGF-β1, therefore ensuing in an extensive minimize in TGF-β brought on transdifferentiation of corneal fibroblasts to myofibroblasts and a discount in fibrosis [162]. Subsequent in vivo research with the aid of the equal crew confirmed therapeutic practicable of AAV-5 primarily based decorin gene remedy with inhibition of fibrosis in rabbit corneal stroma after PRK, besides extensive keratocyte apoptosis or immune cells infiltration. Similarly, non-viral gene remedy the use of polyethylenimine (PEI) NP used to be additionally employed to supply decorin gene safely and correctly in equine corneal cells and minimize fibrosis in vitro [167].

Although TGF-β is central to the procedure of fibrosis, it is additionally necessary for the ordinary wound restoration process. Therefore, greater latest therapeutic methods have been directed closer to the downstream objectives of TGF-β signaling. PEI NP – mediated transport of soluble TGF-β receptor two gene (sTGFBRII) fused to the Fc component of human IgG confirmed attenuation of TGF-β1 triggered transformation of cultured human corneal fibroblasts to myofibroblasts with no extensive mobile death. This impact may want to perchance be due to sequestering adsorption of TGF-β or via appearing as a dominant-negative receptor [168]. Other objectives of TGF-β signaling encompass cytokines such as hepatocyte boom aspect (HGF) and bone morphogenic protein 7 (BMP7) that inhibit TGF-β endeavor in tissue fibrosis [169, 170], as properly as inhibitory Smad7 protein, a bad regulator of TGF-β signaling [171]. In a mouse mannequin of corneal alkali burn, burned corneas dealt with with Ad-BMP7 confirmed lowered scarring after 20 days as in contrast to the control. The authors in addition tested that the impact was once due to the activation of Smad1/5/8 signaling and partial suppression of the phospho-Smad2 signal, as a consequence counteracting TGF-β outcomes [172]. Similarly, localized BMP7 gene shipping by means of PEI-conjugated gold NPs inhibited fibrosis with

the aid of counterbalancing TGF-β1 mediated pro-fibrotic Smad signaling in rabbit corneas in a PRK mannequin of fibrosis [50]. The Mohan team additionally used aggregate remedy with BMP+HGF to deal with corneal fibrosis and repair transparency in rabbits after alkali harm with the aid of two impartial pathways. BMP7 acted by means of antagonizing TGF-β and inhibited the activation of new myofibroblasts, whilst HGF promoted apoptosis of set up myofibroblasts. This aggregate remedy triggered minimal ocular toxicity, however this used to be a momentary find out about of three weeks, and long-term results stay to be considered [173]. Wang et al. 2013 additionally confirmed that lentivirus-mediated overexpression of the Smad7 gene mitigated the activation of TGF-β signaling in rat corneas by means of lowering the phosphorylation of Smad2 and the expression of TGF-β2 after PRK surgical treatment [40]. Similarly, in an in vivo rabbit mannequin of corneal fibrosis triggered with the aid of PRK (Fig. 2), AAV-5 mediated Smad7 gene remedy was once secure and high-quality in inhibiting corneal scarring [34].

Apart from the cornea, TGF-β has additionally been centered for some other ocular surface scarring. In a mannequin of aggressive scarring after glaucoma filtration surgery, TGF-β2 AON therapy extensively decreased conjunctival scarring after a single administration and used to be extra strong than AON to TGF-β1, most probably due to the fact TGF-β2 is the predominant TGF-β isoform related with conjunctival scarring [174,175]. Similarly, discount in subconjunctival scarring submit glaucoma filtration surgical procedure in a rabbit mannequin used to be additionally performed by means of inhibiting SPARC (secreted protein, acidic and wealthy in cysteine) that is related with immoderate accumulation of collagen, the usage of positive-charge tuned gelatin hydrogel-based shipping [176]. Other tactics to limit corneal scarring encompass the overexpression of peroxisome proliferator-activated receptor-γ (PPAR-γ) by Ad gene switch to suppress TGF-β1 triggered profibrotic technology of myofibroblasts. Ad-PPAR-γ therapy resulted in the suppression of profibrotic matrix metalloproteinase MMP-9 in macrophages as nicely as of collagen kind I in the presence of TGF-β1. Moreover, it additionally prompted the in vitro proliferation of corneal epithelial cells however no longer of fibroblasts, per chance with the aid of the inhibition of TGF-β1. In addition to the in vitro effects, PPAR-γ overexpression in a mouse mannequin of corneal alkali burns additionally promoted epithelial recovery post-injury with the aid of the mRNA discount of profibrotic MMP and speedy restoration of the basement membrane [177]. Galiacy et al. (2011) avoided atypical collagen deposition via overexpressing MMP-14 by means of a single injection of AAV vector in a mouse mannequin of corneal full-thickness incision [70]. This cure additionally brought about a marked discount in corneal opacity as nicely as lowered expression α-SMA and collagen kind III, however with toxicity at greater doses [70]. Keratocyte proliferation was once centered via topical administration of retroviral gene remedy to express dominant-negative cyclin G1 in excimer laser-induced corneal haze after phototherapeutic keratectomy (PTK) in rabbits. The use of eye drops of mutant cyclin G1 retroviral vector inhibited keratocyte proliferation and notably decreased extraordinary deposition of ECM proteins. The remedy had no poor results on epithelial proliferation, perhaps due to the safety of the limbal location from vector publicity and the negative in vivo transduction effectivity of retroviral dealers in epithelial cells [178]. AAV-mediated expression of the immunomodulatory and anti-inflammatory molecule, human leukocyte antigen-G (HLA-G) in rabbit corneas additionally inhibited α-SMA expression indicating a discount in myofibroblast activation and fibrosis. However, it is uncertain if HLA-G has a direct hyperlink to myofibroblast technology and the impact appears greater probably to be linked to its anti-inflammatory moves [31].

4.2. Corneal Epithelial Wound Healing

Wound recovery of the corneal epithelium is a clinically applicable phenomenon and has been a problem of severa studies. It is a complicated procedure involving cell adhesion, migration, proliferation, differentiation, stratification, and ECM redesigning mediated through a number increase factors, cytokines, and cell signaling activities [179]. Our team has drastically used each viral and non-viral gene remedy for the transport of goal genes in the human diabetic corneas characterised by using gradual epithelial wound healing. The genes with altered expression in diabetic corneas [180, 181] coded for HGF receptor, c-Met, cathepsin F, proteinases and MMP-10. HGF/c-Met signaling performs an essential position in wound healing-related methods of telephone migration, proliferation, and apoptosis in a number of organ systems, such as the cornea [182–184]. Gene microarray evaluation confirmed that HGF used to be upregulated in diabetic corneas with downregulation of c-Met [181]. The impact of c-Met on epithelial wound restoration was once examined the use of c-Met gene transduction with rAd vector in human diabetic organ-cultured corneas. Overexpression of c-Met normalized the expression of diabetic markers, laminin, nidogen-1, and integrin α3β1, and influenced wound recuperation by using restoring HGF signaling and activation of p38 MAPK pathway [13]. The diabetic corneas additionally have expanded expression of proteinases, cathepsin F and MMP10 [180, 181]. Ad-mediated overexpression of these genes in non-diabetic corneas resulted in diabetes-like modifications [182]. Conversely, rAd-driven shRNA silencing of

cathepsin F and MMP-10 genes extended diabetic epithelial wound recovery with the aid of the activation of EGFR and Akt signaling and additionally restored the expression of diabetic markers integrin α3β1 and nidogen-1, as properly as diabetes-suppressed putative stem phone markers, ΔNp63α, keratin 17, and ABCG2 [109, 183]. Combined remedy with c-Met overexpression used to be even greater environment friendly [109]. rAd gene therapy with c-Met and shRNA to cathepsin F and MMP-10 for wound restoration used to be additionally profitable if solely the limbus harboring epithelial stem cells used to be transduced [110]. This Ad-mediated gene remedy was once abruptly poisonous for limbal epithelial stem cellphone (LESC)enriched fundamental limbal epithelial cellphone (LEC) cultures and resulted in impaired wound recovery [183]. Therefore, a non-viral method was once examined with nanobioconjugates (NBC) developed on herbal polymalic acid (PMLA) scaffold to supply AON to goal cathepsin F, MMP-10, and c-Met inhibiting miR-409 in human diabetic LEC as nicely as human diabetic organ-cultured corneas. The utility of NBC safely and correctly ameliorated diabetes-impaired wound recovery and normalized the LESC and diabetic marker expression [100]. This might also be a promising new method for normalizing wound restoration in diabetic corneas.

Another increase thing that modulates corneal epithelial wound recuperation is the OGF that acts as a poor regulator of epithelial proliferation [184]. Transfection of OGFr antisense the usage of gene gun into rat corneas after central corneal abrasion resulted in fewer epithelial defects and accelerated wound healing, whereas the experience OGFr assemble delayed recovery [83, 84]. Delivery of anti-apoptotic gene bcl-xL has additionally been used to limit phone dying precipitated by means of corneal epithelial debridement [62]. MiRs can additionally be utilized for reaching gene silencing and subsequently can be used to modulate goal genes [179]. Saghizadeh et al. proven that antisense (antagomir) to diabeteselevated miR-146a more suitable corneal wound restoration by the activation of EGFR and p38 signaling and normalized the expression of diabetic and stem mobilephone markers [185, 186]. MiR-155-5p additionally promoted the restore of corneal damage and decreased corneal epithelial permeability in rats with the aid of reducing the expression of myosin mild chain kinase and phosphorylation of myosin mild chain [187]. However, the use of miRs for gene remedy must be totally validated for due to the recognized impact of most miRs on more than one targets.[i]

4.3. Corneal Graft Survival

The cornea is the most typically transplanted tissue in human beings worldwide. Although avascular corneas have the immune privilege [188] a vast quantity of corneal grafts endure allograft rejection after PKP, various between 5 and 40% [189]. There are full-size aspect outcomes with the extended use of systemic immunosuppression to minimize graft rejection [190]. A gene change strategy used to be for that reason used in quite a number fashions by means of introducing immunoregulatory molecules, anti-angiogenic factors, or by means of inhibiting apoptotic pathways in donor and recipient corneas to forestall graft rejection [191].

4.3.1. Immunomodulatory Factors

Overexpression of immunomodulatory boom elements and cytokines has been notably studied to stop corneal graft rejection. Oral et al. (1997) confirmed that it used to be feasible to infect ex vivo human corneas with recombinant Ad to transduce the gene encoding the immunomodulatory CTLA-4 Ig protein to regulate the alloresponse directed in opposition to the graft [192]. They similarly validated drastically extended corneal graft survival after a single administration of this viral vector [193]. Zhou et al. (2010) focused TGF-β1, a mighty immunosuppressive cytokine in the corneal endothelium the use of rAAV prior to transplantation to extend graft survival in a high-risk rat mannequin [194]. Corneal endothelial cells are the most vital goals for graft survival gene amendment due to the irreversible injury brought on to these cells at some stage in rejection due to the fact of their negligible replication ability in humans. Ad-mediated nearby shipping of anti-inflammatory IL-10 gene into donor corneal endothelium right now earlier than transplantation may want to lengthen corneal allograft survival in sheep [195]. In contrast, nearby ex vivo liposome-mediated viral IL-10 gene switch or ex vivo Ad-IL-10 gene switch had no impact on graft survival, however the systemic expression of IL-10 the usage of rAd prolonged graft survival in rats [196]. Lentivirus carrying IL-10 transgene additionally extended ovine orthotopic corneal allograft survival, however no longer as effectively as Ad vector on account of the exceedingly low transgene tiers [197].

More recently, Ad and lentiviral vectors have been used to transduce limbal graft tissue ex vivo with biologically lively IL-10, main to delayed rejection in rats [15]. In addition to IL-10 gene transfer, blockading of pro-inflammatory IL-12 with the aid of nearby overexpression of the IL-12 p40 inhibitory subunit additionally extended sheep corneal graft survival [198]. On the different hand, no prolongation of allogeneic graft survival was once got in rat corneas with the aid of Ad-mediated IL-12 p40 switch [199]. Non-viral Entranster™, an NP vector, was once employed to supply CD25 siRNA that resulted in extended graft survival in rat corneas by means of the upregulation of IL-10 expression [107]. Increased graft survival and inhibition of neovascularization had been found after stromal (before grafting) or anterior chamber (after grafting) injection of a plasmid bearing IL-1 receptor antagonist gene [200]. Inhibiting T mobilephone activation by way of lentiviral gene remedy used to be additionally used to suppress graft rejection. Lentivirus mediated overexpression of programmed phone deathligand 1 (PD-L1) in organ-cultured allogeneic rat corneas reduced CD3+CD8+CD161− and CD3+CD8+CD161+ T cells upon transplantation of modified corneas and decreased inflammatory cytokines IFN-γ and IL-6 ensuing in notably higher graft survival [201].

4.3.2. Pro- and Anti-apoptotic Factors

Corneal endothelial injury precipitated at some point of graft rejection can also be an end result of apoptotic cell dying [202]. Overexpression of the antiapoptotic baculoviral p35 protein in corneal epithelial sheets decreased the priming of T cells in draining lymph nodes after transplantation [203]. Anti-apoptotic genes coding for bcl-xL, bcl-2, survivin, and p35 have been additionally examined in cultured corneal endothelial cells, with the great anti-apoptotic impact acquired with bcl-xL. The respective gene used to be then delivered in the endothelium of donor corneas the usage of lentivirus ensuing in extended survival of allografts in mice [204]. Endothelial phone loss in the course of storage is additionally a motive for donor cornea rejection for transplantation. Therefore, Fuchsluger et al. utilized lentivirus-mediated gene switch of bcl-xL and p35 to effectively lengthen the survival of endothelial cells throughout preservation/storage, which should show really useful for future corneal transplantations [205, 206].

4.4. Corneal Neovascularization

Pathological corneal neovascularization (CNV) motives a loss of corneal transparency and visible acuity and is a main chance element for graft rejection after corneal transplantation and a postoperative complication. Angiogenic privilege, the stability between a low stage of angiogenic and an excessive degree of antiangiogenic factors, has been the key to preserving corneal avascularity [76]. Significant growth has been made in the appreciation of the mechanisms worried in angiogenic privilege, which has led to gene remedy methods the use of transgenic expression of antiangiogenic elements or inactivation of proangiogenic elements by gene silencing [207]. The angiogenic VEGF pathway has been focused via gene switch of VEGF receptors, Flt-1 and Flk-1. Gene switch of soluble Flt-1 the usage of rAAV in cauterized rats [208] and the usage of non-viral micellar nano vector in mice [209] efficaciously inhibited CNV. Recombinant Ad-mediated transport of soluble Flk-1 in cauterized rats had a comparable impact [210]. Recombinant Ad-driven VEGF AON [211] and poly(lactic co-glycolic acid) NP-mediated transport of VEGF-A shRNA [212] had been each in a position to downregulate VEGF and decrease corneal neovascular response. Subconjunctival travelling of lipoplexes carrying the gene encoding GA-binding protein (GABP), a transcription thing that regulates the expression of angiogenic elements VEGF and roundabout4 (Robo4), delayed CNV in a mouse mannequin of corneal injury, however the impact was once quite short-lived [213]. In addition to these, endostatin and angiostatin, two antiangiogenic elements that act via the inhibition of VEGF and FGF-2, have been employed for CNV gene therapy. AAV-mediated endostatin and angiostatin subconjunctival gene transport (Fig. 6) inhibited CNV in mouse and rat corneal damage fashions [214, 215]. Equine infectious anemia virus (EIAV)-based lentiviral vector harboring each endostatin and angiostatin genes suppressed CNV and lowered immune telephone infiltration and corneal opacification in a rabbit mannequin of corneal rejection [39]. Recombinant retroviral vectors encoding more than one murine antiangiogenic genes (soluble Flk-1, Tie2, endostatin) inhibited the proliferation and migration of human umbilical vein endothelial cells (HUVEC) in vitro and decreased CNV in mice [216].

The immunomodulatory and anti-inflammatory molecule, human leukocyte antigen-G (HLA-G) has additionally been delivered with the aid of an AAV vector to decrease injury-induced CNV and immune telephone infiltration in rabbits [31]. Although gene remedy for CNV has been substantially investigated, solely aganirsen AON that inhibits insulin receptor substrate-1 expression has been correctly examined clinically and safely administered as eye drops ensuing in the inhibition of keratitis-induced CNV, lowering the want for corneal transplantation [95, 96].

Other therapeutic processes consist of overexpression of miR-204 (downregulated in neovascularized mouse corneas) that attenuated CNV in injured mouse cornea by a couple of pathways (Angpt1/Tie2/PI3K/Akt) [30]. Synthetic amphiphile INTeraction-18 (SAINT-18) plasmid carrying anti-angiogenic pigment epithelium-derived component gene inhibited CNV in an experimental model of rat corneal angiogenesis [217]. More recently, downregulation of proangiogenic MMP-9 used to be performed the usage of lipid NPs to supply shRNA for treating CNV [218]. Similarly, a cholesterol-modified siRNA transport gadget concentrated on stromal cell-derived issue 1 (SDF-1) inhibited neovascularization and HUVEC proliferation by means of inhibiting Akt signaling [219]. Overall, gene remedy to inhibit pathological corneal neovascularization through performing on a quantity of promising objectives may additionally produce newly accredited capsules in the close to future.

4.5. Genetic Corneal Dystrophies

Corneal dystrophies are heritable revolutionary issues that can alter the corneal shape, reduce corneal transparency, and sooner or later reason imaginative and prescient loss in each eyes. There are 29 issues characterised as corneal dystrophies, with 22 problems related with genetic abnormalities, which may additionally maybe be amenable to remedy by means of gene remedy [27]. Existing remedies for extreme corneal dystrophies that are automatically used these days are keratoplasty and corneal transplantation. However, there are drawbacks of these procedures, such as graft rejection, procedural complications, and a restricted furnish of wholesome donor corneas outdoor of the United States. Additionally, there is a huge vary of phenotypes and severity of medical signs and symptoms related with corneal dystrophies. Therefore, different present day treatments, such as ointments and eye drops, do no longer tackle the individualized worries and are mostly symptomatic [220]. By figuring out the underlying genetic defect, gene remedy can emerge as a higher and extra individualized intervention in treating corneal dystrophies.

Most corneal dystrophies showcase a monogenic or Mendelian sample of inheritance, which makes them best candidates for gene therapy. Gene remedy interventions that are capable to knock out mutant genes or block translation of mutant proteins might also be extra superb in autosomal dominant corneal dystrophies, such as Meesmann epithelial corneal dystrophy (MECD), lattice corneal dystrophy kind 1 (LCD1), granular corneal dystrophy (GCD), and FECD. Allele-specific siRNAs and the CRISPR-Cas9 machine already emerged as possible remedy selections for the prevention of the MECD and LCD corneal pathology.In a find out about the use of corneal limbal biopsy from sufferers with MECD, siRNA remedy absolutely blocked endogenous mutant keratin 12 alleles in limbal epithelial mobilephone cultures with no impact on wild-type allele expression. This approach, in conjunction with an appropriate siRNA shipping vector, may additionally have the plausible to deal with people with MECD [106]. A later find out about by using the identical team the use of the CRISPR-Cas9 gadget confirmed in vivo gene modifying of a heterozygous disease causing SNP that effects in a novel protospacer adjoining motif to goal keratin 12 mutation [221]. Recently, this team has designed a corneal bioluminescence K12-luciferase multitarget knock-in mouse that will assist the in vivo real--time find out about of siRNA shipping of mutant K12 allele expression for the prevention of MECD pathology [222].

In an ex vivo mannequin of limbal biopsy from sufferers with LCD1, a siRNA unique to the TGF-β-induced (TGFBI) gene allele used to be proven to effectively suppress the mutant allele, indicating this promising gene silencing method in different TFBI-associated corneal dystrophies as properly [223]. A comparable strategy the use of the CRISPR-Cas9 gadget also confirmed interference of the TGFBI mutant alleles in corneal dystrophy models. However, this strategy lacked the capability to distinguish between wild-type and mutant alleles, as a consequence yielding cleavage of the wild-type replica as properly [224]. GCD is any other corneal dystrophy that entails the mutant TGFBI gene; however, it is manifested as a couple of discrete and irregular formed granular opacities deposited in the corneal stroma. The CRISPRCas9 machine used to be additionally utilized to right the TFGBI mutation in GCD patient-derived predominant corneal keratocytes in vitro with the use of a homology-directed restore [225]. However, a difficulty of this find out about used to be modest correction effectivity ranging from 20.6% in heterozygous cells to 41.3% in homozygous cells. Although the CRISPR-Cas9 gene-editing device may also in the end get into sanatorium to deal with hereditary corneal dystrophies [226, 227], greater work wishes to be carried out to expand its efficacy. FECD is the most frequent posterior corneal dystrophy and is characterised with the aid of guttae (extracellular matrix outgrowths of Descemet's membrane), endothelial cellphone degeneration, and stromal edema [7]. Most instances of FECD (70%) are induced with the aid of a TCF4 trinucleotide repeat growth main to good sized modifications in mRNA splicing. However, uncommon FECD instances are related with mutations in other genes, inclusive of COL8A2, SLC4A11, ZEB1, and LOXHD1 [206]. Emerging statistics factor to the validity of gene remedy in FECD by means of focused on the TCF4 gene. In a FECD subject-derived corneal endothelial mobile model, the efficiency of AON remedy in ameliorating TCF4 repeat expansion-mediated toxicity used to be proven [101].

Additionally, the CRISPR-Cas9 device confirmed promise for FECD treatment. Recently, Rong et al. (2020) delivered dCas9 and TCF4 repeat-targeting single information RNA to patient-derived corneal endothelial cells via lipofection or lentiviral transduction. This therapy resulted in a significant, 10-fold discount in the proportion of cells with poisonous nuclear RNA foci and the quantity of foci per a hundred cells [228]. With tremendous transport to corneal endothelial cells, this technique may also end up a workable cure for FECD, lowering the necessity for corneal transplantation.

4.6. Herpetic Keratitis

Herpetic keratitis is a viral contamination precipitated by way of the herpes simplex virus kind 1 (HSV-1), normally recognized as the bloodless sore virus, main to corneal blindness and corneal graft rejection [98]. After infection, it develops indefinite latency in the trigeminal ganglion and then strikes to different systems, consisting of the cornea [229, 230]. Corticosteroids are oftentimes used to deal with HSV-1 contamination [231], however gene remedy tactics have additionally been examined in the cornea by using concentrated on corneal irritation and neovascularization, and greater recently, by using immediately focused on the HSV-1 genome.Several inflammatory mediators have been focused to forestall HSV-1 mediated corneal inflammation. Topical administration of plasmid DNA with IL-4, IL-10, IL-12, and IL-18 genes has proven a discount in inflammatory lesion severity in quite a number research [68, 232– 234]. Similarly, different mouse research confirmed that topical transport of interferon (IFN)α1, IFN-β, and IFN-γ via a plasmid DNA 12–24 hours earlier than contamination resulted in expanded survival [235–239].

Some research had been aimed at growing a vaccine the usage of gene remedy to introduce plasmid DNA encoding HSV-1 glycoproteins (gB, gC, gD, gE, and gI) to elicit humoral and cell immunity with various consequences on herpetic keratitis [240, 241]. Inoue et al. used NP-mediated transport of IL-21 and gD vaccine and inhibited herpetic keratitis in HSV-1 contaminated mice, with the aggregate displaying higher effects than gD by myself [242–244]. Although decreasing infection and growing survival is recommended in treating herpetic keratitis, it does no longer honestly therapy the disease. For this reason, in latest years, the center of attention has shifted to focused on the HSV-1 genome for degradation the use of ribozymes [245], AONs [246], siRNA [247], aptamers [248], and homing endonucleases [249].

4.7. Glaucoma

Glaucoma is regularly related with improved intraocular stress (IOP) triggered by means of accelerated resistance to the drainage of aqueous humor thru the traditional outflow machine comprising the trabecular meshwork (TM) and the Schlemm's canal. It consequences in blindness in greater than 60 million humans worldwide. The TM positioned round the base of the cornea is the desired pharmacological goal for gene remedy in the remedy of glaucoma via decreasing intraocular stress to subsequently retard visible subject loss [250].AAV vectors have been in a position to set off long-term, secure transport of transgenes to the TM of rats and monkeys [251]. Several AAV and Ad had been additionally examined in vitro (in human immortalized TM cellphone lines) and in vivo via intracameral injection in mice, with various outcomes [252]. AAV was once additionally correctly used to supply MMP-3 (decreased in human glaucomatous aqueous humor) gene ensuing in elevated MMP-3 expression and lowered IOP [253]. In a rabbit mannequin of glaucoma filtration surgery, Ad-mediated gene switch of p27KIP1, a member of the cyclin-dependent kinase inhibitor family, decreased IOP and avoided scarring [254]. Ad-mediated gene switch of dominant-negative RhoA improved outflow facility in perfused human anterior phase cultures due to loosening of the cell-substrate and cell-cell attachments in the cells of the outflow pathway [255]. Numerous siRNA-based techniques have been studied for the cure of glaucoma [256]. Silencing of RhoA the usage of siRNA in TM decreased IOP in mice [105]. Similarly, topical administration of siRNA to P2Y(2) purinergic receptor [257] and β2 adrenergic receptors [258] additionally decreased IOP in New Zealand rabbits, which ought to be used for glaucoma treatment. Recent in vivo facts additionally confirmed that AAV2-mediated switch of exoenzyme C3 transferase gene, which inactivates Rho via ADP ribosylation [259], through intracameral injection into mouse and monkey eyes, resulted in morphological adjustments in TM and IOP discount [260]. These effects propose the significance of gene remedy as a promising future therapy of glaucoma.

4.8. Dry Eye Disease

Dry eye disorder is brought on with the aid of poor tear manufacturing by using the lacrimal gland or immoderate evaporation. If left untreated, it can also lead to corneal inflammation and scarring [261]. Several autoimmune illnesses like Sjögren's syndrome, rheumatoid arthritis, and lupus erythematosus are related with dry eye ailment [262]. Ad-mediated switch of TNF-α inhibitor gene in a caused autoimmune dacryoadenitis rabbit mannequin restored tear manufacturing to everyday tiers and decreased corneal floor defects [263]. This crew additionally confirmed that AAV-mediated transport of IL-10 gene suppressed lacrimal gland

immunopathology with diminished quantity of CD18+ cells and a smaller ratio of CD4/CD8 cells [264]. Lacrimal gland fluid motion used to be additionally restored with the aid of aquaporin-1 gene remedy the usage of an AAV vector administered to the submandibular glands in a mouse mannequin of Sjögren's syndrome. The impact was once due to BMP6 rules [265]. MUC5AC, glycosylated mucin that acts as an ocular floor lubricant, is reduced in keratoconjunctivitis sicca, Sjögren's syndrome, and Stevens-Johnson syndrome [266–269]. Cationized gelatin-based NPs have been used to supply the MUC5AC gene ensuing in restoration of MUC5AC degrees and discount in infection with no vascularization or edema, and elevated tear production in a mouse mannequin of dry eye [270]. These promising information warrant similarly translation of dry eye gene remedy into human trials.

4.9. Mucopolysaccharidosis

Mucopolysaccharidosis (MPS) is an autosomal recessive ailment affecting lysosomal storage ensuing in corneal clouding [21]. In the eyes, the lack of lysosomal enzymes motives an accumulation of glycosaminoglycans (GAG). Based on the enzyme defect and ailment severity, seven one of a kind types of MPS have been diagnosed [272, 273]. Administration of Ad expressing human β-glucuronidase gene, which is mutated in MPS-VII, into the anterior chamber or intrastromal vicinity of the affected mouse corneas correctly handled corneal clouding in MPS-VII [274]. A comparable impact was once additionally considered in a mannequin of canines MPS VII [275]. Recombinant AAV-mediated transduction of the gene encoding α-L-iduronidase gene (IUDA) (mutation in this gene reasons MPS-I) was once capable to repair IUDA characteristic in the cornea with an attainable to reverse MPS-associated corneal blindness [33]. Overall, gene remedy seems to have outstanding plausible in mitigating the ocular impact in a number of varieties of MPS.

4.10. Aniridia

Aniridia is a congenital circumstance ensuing in partial or whole iris hypoplasia/loss and most many times influences each eyes. It may also end result from an ocular trauma or, extra frequently, from a genetic disease that might also lead in the cornea to aniridia-associated keratopathy (AAK) [276–278]. Most instances of aniridia existing with haploinsufficiency truncating mutation in the PAX6 gene, though about one-third of instances are related with chromosomal rearrangements at the 11(13p) area [277, 278]. CRISPR-Cas9 gadget has been these days examined for gene remedy correction of PAX6 mutations. A novel aniridia mouse mannequin containing the small eye PAX6 mutation (Sey) additionally determined in sufferers used to be used for in vitro and in vivo germline correction of the Sey mutation by means of the CRISPR-Cas9 system. This correction used to be profitable in vitro at about 35%, and in vivo, it resulted in the restoration of FLAG-tagged PAX6 protein expression and normalized eyes [279]. CRISPR-Cas9 technological know-how has additionally been used to introduce a heterozygous nonsense mutation discovered in AAK patients' PAX6 gene into cultured human telomerase-immortalized LECs. This resulted in a massive practical trade negatively affecting cellphone proliferation, migration and detachment. The strange phenotype should be rescued through the introduction of recombinant PAX6 protein [280]. CRISPR-Cas9 technological know-how has for that reason enabled the improvement of cell and animal fashions for aniridia and the rescue of mutant phenotype as a proof-of-concept cure to deal with related imaginative and prescient loss.

II. CONCLUSION

In the closing decade, the gene remedy area has benefited from a range of vectors and transport methods, and essential efficacy information have been got in animal models and humans. Currently, many of these more recent strategies have been examined in the anterior eye phase as nicely and produced promising results. Although pretty a few gene remedy tablets have been accepted recently for human use, the most important advances in this subject pertaining to the anterior eye phase relate solely to animal models or in vitro studies. The translation into the medical institution is sincerely lagging at the back of the traits for the posterior segment, along with retinal ailments and age-related macular degeneration. In the anterior segment, topical AON aganirsen for corneal neovascularization has achieved phase III medical trial in Europe with advantageous consequences [95]. Another scientific trial in China for the use of CRISPR-Cas9 gene-editing remedy to deal with refractory viral keratitis is presently recruiting patients. Although simply a few, these trends supply hope for the translation of gene remedy for the anterior section stipulations into the health center in the close to future.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

Authors have equally participated and shared every item of the work.

REFERENCES

[1]- Anguela, Xavier M., and Katherine A. High. "Entering the modern era of gene therapy." *Annual review of medicine* 70 (2019): 273-288.

[2]- Wirth, Thomas, Nigel Parker, and Seppo Ylä-Herttuala. "History of gene therapy." *Gene* 525.2 (2013): 162-169.

[3]- Dunbar, Cynthia E., et al. "Gene therapy comes of age." *Science* 359.6372 (2018): eaan4672.

[4]- Booth, Claire, et al. "Gene therapy for primary immunodeficiency." *Human Molecular Genetics* 28.R1 (2019): R15-R23.

[5]- Bennett, Jean, and Albert M. Maguire. "Gene therapy for ocular disease." *Molecular Therapy* 1.6 (2000): 501-505.

[6]- Borrás, Teresa. "Recent developments in ocular gene therapy." *Experimental eye research* 76.6 (2003): 643-652.

[7]- Di Iorio, Enzo, et al. "New frontiers of corneal gene therapy." *Human Gene Therapy* 30.8 (2019): 923-945.

[8]- Ljubimov, Alexander V. "Overview of gene therapy in anterior segment." *Investigative Ophthalmology & Visual Science* 60.9 (2019): 1037-1037.

[9]- Mohan, Rajiv R., Lynn M. Martin, and Nishant R. Sinha. "Novel insights into gene therapy in the cornea." *Experimental eye research* 202 (2021): 108361.

[10]- Shirley, Jamie L., et al. "Immune responses to viral gene therapy vectors." *Molecular Therapy* 28.3 (2020): 709-722.

[11]- Volpers, Christoph, and Stefan Kochanek. "Adenoviral vectors for gene transfer and therapy." *The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications* 6.S1 (2004): S164-S171.

[12]- Mohan, Rajiv R., Jason T. Rodier, and Ajay Sharma. "Corneal gene therapy: basic science and translational perspective." *The ocular surface* 11.3 (2013): 150-164.

[13]- Saghizadeh, Mehrnoosh, et al. "Normalization of wound healing and diabetic markers in organ cultured human diabetic corneas by adenoviral delivery of c-Met gene." *Investigative ophthalmology & visual science* 51.4 (2010): 1970-1980.

[14]- Lai, Chooi-May, et al. "Inhibition of angiogenesis by adenovirus-mediated sFlt-1 expression in a rat model of corneal neovascularization." *Human gene therapy* 12.10 (2001): 1299-1310.

[15]- Kaufmann, Claude, et al. "Interleukin-10 gene transfer in rat limbal transplantation." *Current eye research* 42.11 (2017): 1426-1434.

[16]- Bessis, N., F. J. GarciaCozar, and M. C. Boissier. "Immune responses to gene therapy vectors: influence on vector function and effector mechanisms." *Gene therapy* 11.1 (2004): S10-S17.

[17]- Kochanek, Stefan. "High-capacity adenoviral vectors for gene transfer and somatic gene therapy." *Human gene therapy*10.15 (1999): 2451-2459.

[18]- Boutin, Sylvie, et al. "Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors." *Human gene therapy*21.6 (2010): 704-712.

[19]- Hareendran, Sangeetha, et al. "Adeno-associated virus (AAV) vectors in gene therapy: immune challenges and strategies to circumvent them." *Reviews in medical virology* 23.6 (2013): 399-413.

[20]- Mingozzi, Federico, and Katherine A. High. "Immune responses to AAV vectors: overcoming barriers to successful gene therapy." *Blood, The Journal of the American Society of Hematology* 122.1 (2013): 23-36.

[21]- Martino, Ashley T., and David M. Markusic. "Immune response mechanisms against AAV vectors in animal models." *Molecular Therapy-Methods & Clinical Development* 17 (2020): 198-208.

[22]- Naso, Michael F., et al. "Adeno-associated virus (AAV) as a vector for gene therapy." *BioDrugs* 31.4 (2017): 317-334.

[23]- Mohan, Rajiv R., et al. "Targeted decorin gene therapy delivered with adeno-associated virus effectively retards corneal neovascularization in vivo." *PloS one* 6.10 (2011): e26432.

[24]- Sharma, Ajay, et al. "Polyethylenimine-conjugated gold nanoparticles: Gene transfer potential and low toxicity in the cornea." *Nanomedicine: Nanotechnology, Biology and Medicine* 7.4 (2011): 505-513.

[25]- Liu, J., et al. "Different tropism of adenoviruses and adeno-associated viruses to corneal cells: implications for corneal gene therapy." *Molecular Vision* 14 (2008): 2087.

[26]- Song, Liujiang, et al. "Serotype survey of AAV gene delivery via subconjunctival injection in mice." *Gene Therapy* 25.6 (2018): 402-414.

[27]- Bastola, Prabhakar, et al. "Adeno-associated virus mediated gene therapy for corneal diseases." *Pharmaceutics* 12.8 (2020): 767.

[28]- Tarallo, Valeria, et al. "Inhibition of choroidal and corneal pathological neovascularization by Plgf1-de gene transfer." *Investigative Ophthalmology & Visual Science* 53.13 (2012): 7989-7996.

[29]- Saint-Geniez, Mali, et al. "Endogenous VEGF is required for visual function: evidence for a survival role on Müller cells and photoreceptors." *PloS one* 3.11 (2008): e3554.

[30]- Lu, Yi, et al. "Transcriptome profiling of neovascularized corneas reveals miR-204 as a multi-target biotherapy deliverable by rAAVs." *Molecular Therapy-Nucleic Acids* 10 (2018): 349-360.

[31]- Hirsch, Matthew L., et al. "AAV vector-meditated expression of HLA-G reduces injury-induced corneal vascularization, immune cell infiltration, and fibrosis." *Scientific Reports* 7.1 (2017): 17840.

[32]- Parker, Douglas GA, et al. "The potential of viral vector-mediated gene transfer to prolong corneal allograft survival." *Current gene therapy* 9.1 (2009): 33-44.

[33]- Vance, Melisa, et al. "AAV gene therapy for MPS1-associated corneal blindness." *Scientific reports* 6.1 (2016): 22131.

[34]- Gupta, Suneel, et al. "Targeted AAV5-Smad7 gene therapy inhibits corneal scarring in vivo." *PLoS One* 12.3 (2017): e0172928.

[35]- Zhou, Lianhong, et al. "Effect of recombinant adeno-associated virus mediated transforming growth factor-beta1 on corneal allograft survival after high-risk penetrating keratoplasty." *Transplant Immunology* 28.4 (2013): 164-169.

[36]- Alvarez-Rivera, Fernando, et al. "Controlled release of rAAV vectors from APMA-functionalized contact lenses for corneal gene therapy." *Pharmaceutics* 12.4 (2020): 335.

[37]- Cockrell, Adam S., and Tal Kafri. "Gene delivery by lentivirus vectors." *Molecular biotechnology* 36 (2007): 184-204.

[38]- Sakuma, Toshie, Michael A. Barry, and Yasuhiro Ikeda. "Lentiviral vectors: basic to translational." *Biochemical Journal* 443.3 (2012): 603-618.

[39]- Parker, Maria, et al. "Suppression of neovascularization of donor corneas by transduction with equine infectious anemia virus-based lentiviral vectors expressing endostatin and angiostatin." *Human Gene Therapy* 25.5 (2014): 408-418.

[40]- Wang, Ti, et al. "Inhibition of corneal fibrosis by Smad7 in rats after photorefractive keratectomy." *Chinese medical journal* 126.08 (2013): 1445-1450.

[41]- Moreira, Pedro Bertino, et al. "Limbal transplantation at a tertiary hospital in Brazil: a retrospective study." *Arquivos Brasileiros DE Oftalmologia* 78 (2015): 207-211.

[42]- Ramamoorth, Murali, and Aparna Narvekar. "Non viral vectors in gene therapy-an overview." *Journal of clinical and diagnostic research: JCDR* 9.1 (2015): GE01.

[43]- Hardee, Cinnamon L., et al. "Advances in non-viral DNA vectors for gene therapy." *Genes* 8.2 (2017): 65.

[44]- Kampik, D., R. R. Ali, and D. F. P. Larkin. "Experimental gene transfer to the corneal endothelium." *Experimental eye research* 95.1 (2012): 54-59.

[45]- Reyes-Sandoval, Arturo, and Hildegund CJ Ertl. "CpG methylation of a plasmid vector results in extended transgene product expression by circumventing induction of immune responses." *Molecular Therapy* 9.2 (2004): 249-261.

[46]-Krieg, Arthur M., et al. "Sequence motifs in adenoviral DNA block immune activation by stimulatory CpG motifs." Proceedings of the National Academy of Sciences 95.21 (1998): 12631-12636.

[47]- Yew, Nelson S., et al. "Reduced inflammatory response to plasmid DNA vectors by elimination and inhibition of immunostimulatory CpG motifs." Molecular therapy 1.3 (2000): 255-262.

[48]- de Wolf, Holger K., et al. "Plasmid CpG depletion improves degree and duration of tumor gene expression after intravenous administration of polyplexes." Pharmaceutical research 25 (2008): 1654-1662.

[49]- Lee, Handule, and Kwangsik Park. "In vitro cytotoxicity of zinc oxide nanoparticles in cultured statens seruminstitut rabbit cornea cells." Toxicological Research 35 (2019): 287-294.

[50]- Tandon, Ashish, et al. "BMP7 gene transfer via gold nanoparticles into stroma inhibits corneal fibrosis in vivo." PloS one 8.6 (2013): e66434.

[51]- Masse, Florence, et al. "Synthesis of ultrastable gold nanoparticles as a new drug delivery system." Molecules 24.16 (2019): 2929.

[52]- Tartaj, Pedro, et al. "The preparation of magnetic nanoparticles for applications in biomedicine." Journal of physics D: Applied physics 36.13 (2003): R182.

[53]- Cornell, Lauren E., et al. "Magnetic nanoparticles as a potential vehicle for corneal endothelium repair." Military medicine 181.suppl_5 (2016): 232-239.

[54]- Xia, Xin, et al. "Magnetic human corneal endothelial cell transplant: delivery, retention, and short-term efficacy." Investigative Ophthalmology & Visual Science 60.7 (2019): 2438-2448.

[55]- Moysidis, Stavros N., et al. "Magnetic field-guided cell delivery with nanoparticle-loaded human corneal endothelial cells." Nanomedicine: Nanotechnology, Biology and Medicine 11.3 (2015): 499-509.

[56]- Tong, Yaw-Chong, et al. "Eye drop delivery of nano-polymeric micelle formulated genes with cornea-specific promoters." The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications 9.11 (2007): 956-966.

[57]- Cholkar, Kishore, et al. "Optimization of dexamethasone mixed nanomicellar formulation." AAPS PharmSciTech 15 (2014): 1454-1467.

[58]- de Redín, Inés Luis, et al. "In vivo effect of bevacizumab-loaded albumin nanoparticles in the treatment of corneal neovascularization." Experimental Eye Research 185 (2019): 107697.

[59]- Jiang, Min, et al. "Cationic core–shell liponanoparticles for ocular gene delivery." Biomaterials 33.30 (2012): 7621-7630.

[60]- Ljubimova, Julia Y., et al. "Covalent nano delivery systems for selective imaging and treatment of brain tumors." Advanced drug delivery reviews 113 (2017): 177-200.

[61]- Kopeček, Jindřich, and Jiyuan Yang. "Polymer nanomedicines." Advanced drug delivery reviews 156 (2020): 40-64.

[62]- Tong, Yaw-Chong, et al. "Polymeric micelle gene delivery of bcl-xL via eye drop reduced corneal apoptosis following epithelial debridement." Journal of Controlled Release 147.1 (2010): 76-83.

[63]- Kalomiraki, Marina, Kyriaki Thermos, and Nikos A. Chaniotakis. "Dendrimers as tunable vectors of drug delivery systems and biomedical and ocular applications." International journal of nanomedicine (2016): 1-12.

[64]- Holden, Christopher A., et al. "Polyamidoamine dendrimer hydrogel for enhanced delivery of antiglaucoma drugs." Nanomedicine: nanotechnology, biology and medicine 8.5 (2012): 776-783.

[65]- Soiberman, Uri, et al. "Subconjunctival injectable dendrimer-dexamethasone gel for the treatment of corneal inflammation." Biomaterials 125 (2017): 38-53.

[66]- Yang, Xiucheng, et al. "A novel dendrimer-based complex co-modified with cyclic RGD hexapeptide and penetratin for noninvasive targeting and penetration of the ocular posterior segment." Drug Delivery 26.1 (2019): 989-1001.

[67]- Maloy, Stanley, and Kelly Hughes, eds. Brenner's encyclopedia of genetics. Academic Press, 2013.

[68]- Kim, Bumseok, et al. "Application of plasmid DNA encoding IL-18 diminishes development of herpetic stromal keratitis by antiangiogenic effects." The Journal of Immunology 175.1 (2005): 509-516.

[69]- Jani, Pooja D., et al. "Nanoparticles sustain expression of Flt intraceptors in the cornea and inhibit injury-induced corneal angiogenesis." Investigative ophthalmology & visual science 48.5 (2007): 2030-2036.

[70]- Galiacy, S. D., et al. "Matrix metalloproteinase 14 overexpression reduces corneal scarring." Gene therapy 18.5 (2011): 462-468.

[71]- He, Zhiguo, et al. "Ex vivo gene electrotransfer to the endothelium of organ cultured human corneas." Ophthalmic research 43.1 (2009): 43-55.

[72]- Blair-Parks, Kathleen, Bonnie C. Weston, and David A. Dean. "High-level gene transfer to the cornea using electroporation." The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications 4.1 (2002): 92-100.

[73]- Rosazza, Christelle, et al. "Gene electrotransfer: a mechanistic perspective." Current gene therapy 16.2 (2016): 98-129.

[74]- Yu, Wen-Zhen, et al. "Gene transfer of kringle 5 of plasminogen by electroporation inhibits corneal neovascularization." Ophthalmic research 35.5 (2003): 239-246.

[75]- Wells, D. J. "Gene therapy progress and prospects: electroporation and other physical methods." Gene therapy 11.18 (2004): 1363-1369.

[76]- Liu, Siyin, et al. "Gene-based antiangiogenic applications for corneal neovascularization." Survey of ophthalmology 63.2 (2018): 193-213.

[77]- Berdugo, M., et al. "Delivery of antisense oligonucleotide to the cornea by iontophoresis." Antisense and Nucleic Acid Drug Development 13.2 (2003): 107-114.

[78]- Sekijima, Hidehisa, et al. "Characterization of ocular iontophoretic drug transport of ionic and non-ionic compounds in isolated rabbit cornea and conjunctiva." Biological and Pharmaceutical Bulletin 39.6 (2016): 959-968.

[79]- Vinciguerra, Paolo, et al. "Iontophoresis-assisted corneal collagen cross-linking with epithelial debridement: preliminary results." BioMed Research International 2016 (2016).

[80]- Bouheraoua, Nacim, et al. "Three different protocols of corneal collagen crosslinking in keratoconus: conventional, accelerated and iontophoresis." JoVE (Journal of Visualized Experiments) 105 (2015): e53119.

[81]- Bikbova, Guzel, and Mukharram Bikbov. "Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin." Acta ophthalmologica 92.1 (2014): e30-e34.

[82]- Zhang, Er-Ping, et al. "Minimizing side effects of ballistic gene transfer into the murine corneal epithelium." Graefe's archive for clinical and experimental ophthalmology 240 (2002): 114-119.

[83]- Zhang, Er-Ping, et al. "Minimizing side effects of ballistic gene transfer into the murine corneal epithelium." Graefe's archive for clinical and experimental ophthalmology 240 (2002): 114-119.

[84]- Zagon, Ian S., et al. "Particle-mediated gene transfer of opioid growth factor receptor cDNA regulates cell proliferation of the corneal epithelium." Cornea 24.5 (2005): 614-619.

[85]- SA, König Merediz, et al. "Ballistic transfer of minimalistic immunologically defined expression constructs for IL4 and CTLA4 into the corneal epithelium in mice after orthotopic corneal allograft transplantation." Graefe's Archive for Clinical and Experimental Ophthalmology= Albrecht von Graefes Archiv fur Klinische und Experimentelle Ophthalmologie 238.8 (2000): 701-707.

[86]- Müller, Anja, et al. "Influence of ballistic gene transfer on antigen-presenting cells in murine corneas." Graefe's archive for clinical and experimental ophthalmology 240 (2002): 851-859.

[87]- Mohan, Rajiv R., et al. "Gene therapy in the cornea." Progress in retinal and eye research 24.5 (2005): 537-559.

[88]- Mohan, Rajiv R., et al. "Gene therapy in the cornea: 2005–present." Progress in retinal and eye research 31.1 (2012): 43-64.

[89]- Bemelmans, A. P., Y. Arsenijevic, and F. Majo. "Efficient lentiviral gene transfer into corneal stroma cells using a femtosecond laser." Gene therapy 16.7 (2009): 933-938.

[90]- Jumelle, Clotilde, et al. "Delivery of macromolecules into the endothelium of whole ex vivo human cornea by femtosecond laser-activated carbon nanoparticles." British Journal of Ophthalmology 100.8 (2016): 1151-1156.

[91]- Toropainen, Elisa, et al. "Corneal epithelium as a platform for secretion of transgene products after transfection with liposomal gene eyedrops." The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications 9.3 (2007): 208-216.

[92]- Dannowski, Haike, et al. "Lipid-mediated gene transfer of acidic fibroblast growth factor into human corneal endothelial cells." Experimental eye research 80.1 (2005): 93-101.

[93]- Rossi, Andrea, et al. "Genetic compensation induced by deleterious mutations but not gene knockdowns." Nature 524.7564 (2015): 230-233.

[94]- Scoles, D. R., E. V. Minikel, and S. M. Pulst. "Antisense oligonucleotides: A primer. Neurol Genet 5: e323." (2019).

[95]- Cursiefen, Claus, et al. "Aganirsen antisense oligonucleotide eye drops inhibit keratitis-induced corneal neovascularization and reduce need for transplantation: the I-CAN study." Ophthalmology 121.9 (2014): 1683-1692.

[96]- Cursiefen, Claus, et al. "Aganirsen antisense oligonucleotide eye drops inhibit keratitis-induced corneal neovascularization and reduce need for transplantation: the I-CAN study." Ophthalmology 121.9 (2014): 1683-1692.

[97]- Wasmuth, Susanne, et al. "Topical treatment with antisense oligonucleotides targeting tumor necrosis factor-α in herpetic stromal keratitis." Investigative ophthalmology & visual science 44.12 (2003): 5228-5234.

[98]- Elbadawy, Hossein Mostafa, et al. "Targeting herpetic keratitis by gene therapy." Journal of ophthalmology 2012 (2012).

[99]- Hu, Jiaxin, et al. "Oligonucleotides targeting TCF4 triplet repeat expansion inhibit RNA foci and mis-splicing in Fuchs' dystrophy." Human molecular genetics 27.6 (2018): 1015-1026.

[100]- Kramerov, Andrei A., et al. "Novel nanopolymer RNA therapeutics normalize human diabetic corneal wound healing and epithelial stem cells." Nanomedicine: Nanotechnology, Biology and Medicine 32 (2021): 102332.

[101]- Zarouchlioti, Christina, et al. "Antisense therapy for a common corneal dystrophy ameliorates TCF4 repeat expansion-mediated toxicity." The American Journal of Human Genetics 102.4 (2018): 528-539.

[102]- Gibson, Daniel J., Sonal S. Tuli, and Gregory S. Schultz. "Dual-phase iontophoresis for the delivery of antisense oligonucleotides." nucleic acid therapeutics 27.4 (2017): 238-250.

[103]- Chau, Viet Q., et al. "Delivery of antisense oligonucleotides to the cornea." nucleic acid therapeutics 30.4 (2020): 207-214.

[104]- Supe, Shibani, Archana Upadhya, and Kavita Singh. "Role of small interfering RNA (siRNA) in targeting ocular neovascularization: a review." Experimental Eye Research 202 (2021): 108329.

[105]- Liu, Q., et al. "siRNA silencing of gene expression in trabecular meshwork: RhoA siRNA reduces IOP in mice." Current Molecular Medicine 12.8 (2012): 1015-1027.

[106]- Courtney, David G., et al. "siRNA silencing of the mutant keratin 12 allele in corneal limbal epithelial cells grown from patients with Meesmann's epithelial corneal dystrophy." Investigative ophthalmology & visual science 55.5 (2014): 3352-3360.

[107]- Qin, Qin, et al. "Effects of CD25siRNA gene transfer on high-risk rat corneal graft rejection." Graefe's Archive for Clinical and Experimental Ophthalmology 253 (2015): 1765-1776.

[108]- Guzman-Aranguez, A., P. Loma, and J. Pintor. "Small-interfering RNA s (siRNA s) as a promising tool for ocular therapy." British journal of pharmacology 170.4 (2013): 730-747.

[109]- Saghizadeh, Mehrnoosh, et al. "Enhanced wound healing, kinase and stem cell marker expression in diabetic organ-cultured human corneas upon MMP-10 and cathepsin F gene silencing." Investigative ophthalmology & visual science 54.13 (2013): 8172-8180.

[110]- Saghizadeh, Mehrnoosh, et al. "Normalization of wound healing and stem cell marker patterns in organ-cultured human diabetic corneas by gene therapy of limbal cells." Experimental eye research 129 (2014): 66-73.

[111]- Schiroli, Davide, et al. "Effective in vivo topical delivery of siRNA and gene silencing in intact corneal epithelium using a modified cell-penetrating peptide." Molecular Therapy-Nucleic Acids 17 (2019): 891-906.

[112]- Baran-Rachwalska, Paulina, et al. "Topical siRNA delivery to the cornea and anterior eye by hybrid silicon-lipid nanoparticles." Journal of Controlled Release 326 (2020): 192-202.

[113]- Yu, Wenhan, and Zhijian Wu. "Ocular delivery of CRISPR/Cas genome editing components for treatment of eye diseases." Advanced drug delivery reviews 168 (2021): 181-195.

[114]- Wu, Zhijian, Hongyan Yang, and Peter Colosi. "Effect of genome size on AAV vector packaging." Molecular Therapy 18.1 (2010): 80-86.

[115]- Suzuki, Takayuki, et al. "Development of a recombinant adenovirus vector production system free of replication-competent adenovirus by utilizing a packaging size limit of the viral genome." Virus research 158.1-2 (2011): 154-160.

[116]- Balaggan, K. S., and R. R. Ali. "Ocular gene delivery using lentiviral vectors." Gene therapy 19.2 (2012): 145-153.

[117]- Yang, Yang, et al. "A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice." Nature biotechnology 34.3 (2016): 334-338.

[118]- Jain, Ankur, et al. "CRISPR-Cas9–based treatment of myocilin-associated glaucoma." Proceedings of the National Academy of Sciences 114.42 (2017): 11199-11204.

[119]- Holmgaard, Andreas, et al. "In vivo knockout of the Vegfa gene by lentiviral delivery of CRISPR/Cas9 in mouse retinal pigment epithelium cells." Molecular Therapy-Nucleic Acids 9 (2017): 89-99.

[120]- Lai, Yi, Yongping Yue, and Dongsheng Duan. "Evidence for the failure of adeno-associated virus serotype 5 to package a viral genome≥ 8.2 kb." Molecular Therapy 18.1 (2010): 75-79.

[121]- Wang, Sui, et al. "A gene regulatory network controls the binary fate decision of rod and bipolar cells in the vertebrate retina." Developmental cell 30.5 (2014): 513-527.

[122]- Bakondi, Benjamin, et al. "In vivo CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa." Molecular Therapy 24.3 (2016): 556-563.

[123]- Chen, Guojun, et al. "A biodegradable nanocapsule delivers a Cas9 ribonucleoprotein complex for in vivo genome editing." Nature nanotechnology 14.10 (2019): 974-980.

[124]- Wang, Yuyuan, et al. "A pH-responsive silica–metal–organic framework hybrid nanoparticle for the delivery of hydrophilic drugs, nucleic acids, and CRISPR-Cas9 genome-editing machineries." Journal of Controlled Release 324 (2020): 194-203.

[125]- Chacon-Camacho, Oscar Francisco, and Juan Carlos Zenteno. "Review and update on the molecular basis of Leber congenital amaurosis." World Journal of Clinical Cases: WJCC 3.2 (2015): 112.

[126]- Pennesi, Mark E., et al. "Residual electroretinograms in young Leber congenital amaurosis patients with mutations of AIPL1." Investigative ophthalmology & visual science 52.11 (2011): 8166-8173.

[127]- Gao, Jie, Rehan M. Hussain, and Christina Y. Weng. "Voretigene neparvovec in retinal diseases: a review of the current clinical evidence." Clinical Ophthalmology (2020): 3855-3869.

[128]- Jacobson, Samuel G., 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." Archives of ophthalmology 130.1 (2012): 9-24.

[129]- Weleber, Richard G., et al. "Results at 2 years after gene therapy for RPE65-deficient Leber congenital amaurosis and severe early-childhood–onset retinal dystrophy." Ophthalmology 123.7 (2016): 1606-1620.

[130]- Chung, Daniel C., et al. "Novel mobility test to assess functional vision in patients with inherited retinal dystrophies." Clinical & experimental ophthalmology 46.3 (2018): 247-259.

[131]- Bennett, Jean, et al. "Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by RPE65 mutations: a follow-on phase 1 trial." The Lancet 388.10045 (2016): 661-672.

[132]- Bainbridge, James WB, et al. "Long-term effect of gene therapy on Leber's congenital amaurosis." New England Journal of Medicine 372.20 (2015): 1887-1897.

[133]- Russell, Stephen, 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." The Lancet 390.10097 (2017): 849-860.

[134]- Grishanin, Ruslan, et al. "Preclinical evaluation of ADVM-022, a novel gene therapy approach to treating wet age-related macular degeneration." Molecular Therapy 27.1 (2019): 118-129.

[135]- Kiss, Szilárd, et al. "Long-term safety evaluation of continuous intraocular delivery of aflibercept by the intravitreal gene therapy candidate ADVM-022 in nonhuman primates." Translational vision science & technology 10.1 (2021): 34-34.

[136]- Gelfman, Claire M., et al. "Comprehensive preclinical assessment of ADVM-022, an intravitreal anti-VEGF gene therapy for the treatment of neovascular AMD and diabetic macular edema." Journal of Ocular Pharmacology and Therapeutics 37.3 (2021): 181-190.

[137]- Kiss, Szilard, et al. "ADVM-022 Intravitreal Gene Therapy for Neovascular AMD-Results from the Phase 1 OPTIC Study." MOLECULAR THERAPY. Vol. 29. No. 4. 50 HAMPSHIRE ST, FLOOR 5, CAMBRIDGE, MA 02139 USA: CELL PRESS, 2021.

[138]- Khanani, Arshad M., et al. "Phase 1 study of intravitreal gene therapy ADVM-022 for neovascular AMD (OPTIC Trial)." Investigative Ophthalmology & Visual Science 61.7 (2020): 1154-1154.

[139]- Busbee, Brandon, et al. "Phase 1 study of intravitreal gene therapy with ADVM-022 for neovascular AMD (OPTIC Trial)." Investigative Ophthalmology & Visual Science 62.8 (2021): 352-352.

[140]- Kiss, Szilárd, et al. "Analysis of aflibercept expression in NHPs following intravitreal administration of ADVM-022, a potential gene therapy for nAMD." Molecular Therapy-Methods & Clinical Development 18 (2020): 345-353.

[141]- Khanani, Arshad M., et al. "Review of gene therapies for age-related macular degeneration." Eye 36.2 (2022): 303-311.

[142]- Siddiqui, F., A. Aziz, and A. M. Khanani. "Gene therapy for neovascular AMD." Retin. Physician 17 (2020): 36-39.

[143]- Giudice, Giuseppe Lo, Alessandro Galan, and Irene Gattazzo. "Introductory Chapter: Treatment of Medical Retinal Diseases by Surgical Approaches–Mini-Review of the Latest Advances in the Field of Ophthalmology." Medical and Surgical Retina-Recent Innovation, New Perspective, and Applications (2023).

[144]- Khanani, Arshad M., et al. "Review of gene therapies for age-related macular degeneration." Eye 36.2 (2022): 303-311.

[145]- Nozaki, Miho, et al. "Drusen complement components C3a and C5a promote choroidal neovascularization." Proceedings of the National Academy of Sciences 103.7 (2006): 2328-2333.

[146]- Dreismann, Anna K., et al. "Functional expression of complement factor I following AAV-mediated gene delivery in the retina of mice and human cells." Gene Therapy 28.5 (2021): 265-276.

[147]- Waheed, N. K. "FOCUS interim results: GT005 gene therapy phase I/II study for the treatment of geographic atrophy." Angiogenesis, Exudation, and Degeneration (2021).

[148]- Tan, Li Xuan, Kimberly A. Toops, and Aparna Lakkaraju. "Protective responses to sublytic complement in the retinal pigment epithelium." Proceedings of the National Academy of Sciences 113.31 (2016): 8789-8794.

[149]-Khanani, Arshad M., et al. "Review of gene therapies for age-related macular degeneration." Eye 36.2 (2022): 303-311.

[150]- Biosciences, Hemera. "Treatment of advanced dry age related macular degeneration with AAVCAGsCD59. ClinicalTrials. gov." (2020).

[151]- Shah, Anjali R., et al. "Predictors of response to intravitreal anti–vascular endothelial growth factor treatment of age-related macular degeneration." American journal of ophthalmology 163 (2016): 154-166.

[152]- Gaudana, Ripal, et al. "Ocular drug delivery." The AAPS journal 12 (2010): 348-360.

[153]- Yellepeddi, Venkata Kashyap, and Srinath Palakurthi. "Recent advances in topical ocular drug delivery." Journal of Ocular Pharmacology and Therapeutics 32.2 (2016): 67-82.

[154]- Joseph, Rini Rachel, and Subbu S. Venkatraman. "Drug delivery to the eye: what benefits do nanocarriers offer?." Nanomedicine 12.6 (2017): 683-702.

[155]- Gote, Vrinda, et al. "Ocular drug delivery: present innovations and future challenges." Journal of Pharmacology and Experimental Therapeutics 370.3 (2019): 602-624.

[156]- Rafiei, Fojan, Hadi Tabesh, and Farrokh Farzad. "Sustained subconjunctival drug delivery systems: Current trends and future perspectives." International Ophthalmology 40 (2020): 2385-2401.

[157]- Barar, Jaleh, Ali Reza Javadzadeh, and Yadollah Omidi. "Ocular novel drug delivery: impacts of membranes and barriers." Expert opinion on drug delivery 5.5 (2008): 567-581.

[158]- Bourne, Rupert RA, et al. "Magnitude, temporal trends, and projections of the global prevalence of blindness and distance and near vision impairment: a systematic review and meta-analysis." The Lancet Global Health 5.9 (2017): e888-e897.

[159]- World Health Organization. "Visual impairment and blindness." Fact sheet 282 (2009).

[160]- Lovicu, Frank, and Daisy Shu. "Myofibroblast transdifferentiation: The dark force in ocular wound healing and fibrosis." (2017).

[161]- Hinz, Boris. "Myofibroblasts." Experimental eye research 142 (2016): 56-70.

[162]- Mohan, Rajiv R., et al. "Decorin transfection suppresses profibrogenic genes and myofibroblast formation in human corneal fibroblasts." Experimental eye research 91.2 (2010): 238-245.

[163]- Schaefer, Liliana, and Renato V. Iozzo. "Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction." Journal of Biological Chemistry 283.31 (2008): 21305-21309.

[164]- Yamaguchi, Yu, David M. Mann, and Erkki Ruoslahti. "Negative regulation of transforming growth factor-β by the proteoglycan decorin." Nature 346.6281 (1990): 281-284.

[165]- Harper, J. R., et al. "[12] Role of transforming growth factor β and decorin in controlling fibrosis." Methods in Enzymology. Vol. 245. Academic Press, 1994. 241-254.

[166]-Huijun, Wang, et al. "Ex vivo transfer of the decorin gene into rat glomerulus via a mesangial cell vector suppressed extracellular matrix accumulation in experimental glomerulonephritis." Experimental and molecular pathology 78.1 (2005): 17-24.

[167]- Donnelly, Kevin S., et al. "Decorin-PEI nanoconstruct attenuates equine corneal fibroblast differentiation." Veterinary ophthalmology 17.3 (2014): 162-169.

[168]- Sharma, Ajay, et al. "Attenuation of corneal myofibroblast development through nanoparticle-mediated soluble transforming growth factor-β type II receptor (sTGFβRII) gene transfer." Molecular Vision 18 (2012): 2598.

[169]- Wang, Shinong, and Raimund Hirschberg. "BMP7 antagonizes TGF-β-dependent fibrogenesis in mesangial cells." American Journal of Physiology-Renal Physiology 284.5 (2003): F1006-F1013.

[170]- Wang, Shinong, and Raimund Hirschberg. "Bone morphogenetic protein-7 signals opposing transforming growth factor β in mesangial cells." Journal of Biological Chemistry 279.22 (2004): 23200-23206.

[171]- Nakao, Atsuhito, et al. "Identification of Smad7, a TGFβ-inducible antagonist of TGF-β signalling." Nature 389.6651 (1997): 631-635.

[172]- Saika, Shizuya, et al. "Therapeutic effects of adenoviral gene transfer of bone morphogenic protein-7 on a corneal alkali injury model in mice." Laboratory Investigation 85.4 (2005): 474-486.

[173]- Gupta, Suneel, et al. "Novel combination BMP7 and HGF gene therapy instigates selective myofibroblast apoptosis and reduces corneal haze in vivo." Investigative ophthalmology & visual science 59.2 (2018): 1045-1057.

[174]- Cordeiro, M. Francesca, et al. "Transforming growth factor-β1,-β2, and-β3 in vivo: effects on normal and mitomycin c–modulated conjunctival scarring." Investigative ophthalmology & visual science 40.9 (1999): 1975-1982.

[175]- Cordeiro, M. F., et al. "Novel antisense oligonucleotides targeting TGF-β inhibit in vivo scarring and improve surgical outcome." Gene therapy 10.1 (2003): 59-71.

[176]- Chun, Yong Yao, et al. "Positive-charge tuned gelatin hydrogel-siSPARC injectable for siRNA anti-scarring therapy in post glaucoma filtration surgery." Scientific Reports 11.1 (2021): 1470.

[177]- Saika, Shizuya, et al. "Effect of overexpression of pparγ on the healing process of corneal alkali burn in mice." American Journal of Physiology-Cell Physiology 293.1 (2007): C75-C86.

[178]- Behrens, Ashley, et al. "Retroviral gene therapy vectors for prevention of excimer laser-induced corneal haze." Investigative ophthalmology & visual science 43.4 (2002): 968-977.

[179]- Kramerov, Andrei A., et al. "Novel nanopolymer RNA therapeutics normalize human diabetic corneal wound healing and epithelial stem cells." Nanomedicine: Nanotechnology, Biology and Medicine 32 (2021): 102332.

[180]- Saghizadeh, Mehrnoosh, et al. "Overexpression of matrix metalloproteinase-10 and matrix metalloproteinase-3 in human diabetic corneas: a possible mechanism of basement membrane and integrin alterations." The American journal of pathology 158.2 (2001): 723-734.

[181]- Saghizadeh, Mehrnoosh, et al. "Proteinase and growth factor alterations revealed by gene microarray analysis of human diabetic corneas." Investigative ophthalmology & visual science 46.10 (2005): 3604-3615.

[182]- Chmielowiec, Jolanta, et al. "c-Met is essential for wound healing in the skin." The Journal of cell biology 177.1 (2007): 151-162.

[183]- Kramerov, Andrei A., Mehrnoosh Saghizadeh, and Alexander V. Ljubimov. "Adenoviral gene therapy for diabetic keratopathy: effects on wound healing and stem cell marker expression in human organ-cultured corneas and limbal epithelial cells." JoVE (Journal of Visualized Experiments) 110 (2016): e54058.

[184]- Sassani, Joseph W., Patricia J. Mc Laughlin, and Ian S. Zagon. "The Yin and Yang of the Opioid Growth Regulatory System: Focus on Diabetes—The Lorenz E. Zimmerman Tribute Lecture." Journal of diabetes research 2016 (2016).

[185]- Funari, Vincent A., et al. "Differentially expressed wound healing-related microRNAs in the human diabetic cornea." PloS one 8.12 (2013): e84425.

[186]- Winkler, Michael A., et al. "Targeting miR-146a to treat delayed wound healing in human diabetic organ-cultured corneas." PloS one 9.12 (2014): e114692.

[187]- Wang, Feng, et al. "MiRNA-155-5p reduces corneal epithelial permeability by remodeling epithelial tight junctions during corneal wound healing." Current Eye Research 45.8 (2020): 904-913.

[188]- Niederkorn, Jerry Y. "Immune privilege of corneal allografts." Cornea and External Eye Disease: Corneal Allotransplantation, Allergic Disease and Trachoma (2010): 1-12.

[189]-Pleyer, Uwe, and Stephan Schlickeiser. "The taming of the shrew? The immunology of corneal transplantation." Acta ophthalmologica 87.5 (2009): 488-497.

[190]- Ritter, Thomas, Mieszko Wilk, and Mikhail Nosov. "Gene therapy approaches to prevent corneal graft rejection: where do we stand?." Ophthalmic Research 50.3 (2013): 135-140.

[191]- Williams, Keryn A., Claire F. Jessup, and Douglas J. Coster. "Gene therapy approaches to prolonging corneal allograft survival." Expert opinion on biological therapy 4.7 (2004): 1059-1071.

[192]-Oral, H. B., et al. "Ex vivo adenovirus-mediated gene transfer and immunomodulatory protein production in human cornea." Gene therapy 4.7 (1997): 639-647.

[193]-Comer, Richard M., et al. "Effect of administration of CTLA4-Ig as protein or cDNA on corneal allograft survival." Investigative ophthalmology & visual science 43.4 (2002): 1095-1103.

[194]-Zhou, Shi-you, et al. "Inhibition of mouse alkali burn induced-corneal neovascularization by recombinant adenovirus encoding human vasohibin-1." Molecular vision 16 (2010): 1389.

[195]- Hori, Junko, et al. "Immune privilege in corneal transplantation." Progress in retinal and eye research 72 (2019): 100758.

[196]- Amador, Cynthia, et al. "Gene therapy in the anterior eye segment." Current gene therapy 22.2 (2022): 104.

[197]-Verhoeff, Kevin, et al. "Inducible pluripotent stem cells as a potential cure for diabetes." Cells 10.2 (2021): 278.

[198]- Klebe, Sonja, et al. "Prolongation of sheep corneal allograft survival by transfer of the gene encoding ovine IL-12-p40 but not IL-4 to donor corneal endothelium." The Journal of Immunology 175.4 (2005): 2219-2226.

[199]-Salabarria, Ann-Charlott, et al. "Local VEGF-A blockade modulates the microenvironment of the corneal graft bed." American Journal of Transplantation 19.9 (2019): 2446-2456.

[200]-Lu, Xiao-Xiao, and Shao-Zhen Zhao. "Gene-based therapeutic tools in the treatment of cornea disease." Current Gene Therapy 19.1 (2019): 7-19.

[201]- Arsenijevic, Yvan, et al. "Lentiviral vectors for ocular gene therapy." Pharmaceutics 14.8 (2022): 1605.

[202]- Price, Marianne O., et al. "Corneal endothelial dysfunction: Evolving understanding and treatment options." Progress in retinal and eye research 82 (2021): 100904.

[203]- Pastak, Marko, et al. "Gene therapy for modulation of T-cell-mediated immune response provoked by corneal transplantation." Human Gene Therapy 29.4 (2018): 467-479.

[204]- Amador, Cynthia, et al. "Gene therapy in the anterior eye segment." Current gene therapy 22.2 (2022): 104.

[205]- Price, Marianne O., et al. "Corneal endothelial dysfunction: Evolving understanding and treatment options." Progress in retinal and eye research 82 (2021): 100904.

[206]- Maurizi, Eleonora, et al. "Nanoneedles induce targeted siRNA silencing of p16 in the human corneal endothelium." Advanced Science 9.33 (2022): 2203257.

[207]- Arsenijevic, Yvan, et al. "Lentiviral vectors for ocular gene therapy." Pharmaceutics 14.8 (2022): 1605.

[208]- Korecki, Andrea J., et al. "Human MiniPromoters for ocular-rAAV expression in ON bipolar, cone, corneal, endothelial, Müller glial, and PAX6 cells." Gene Therapy 28.6 (2021): 351-372.

[209]- Ruan, Yue, Subao Jiang, and Adrian Gericke. "Age-related macular degeneration: role of oxidative stress and blood vessels." International journal of molecular sciences 22.3 (2021): 1296.

[210]- Kumar, Satheesh, et al. "RNA-targeting strategies as a platform for ocular gene therapy." Progress in Retinal and Eye Research 92 (2023): 101110.

[211]- Han, Haijie, et al. "Polymer-and lipid-based nanocarriers for ocular drug delivery: Current status and future perspectives." Advanced Drug Delivery Reviews (2023): 114770.

[212]-Sharif, Zuhair, and Walid Sharif. "Corneal neovascularization: updates on pathophysiology, investigations & management." Romanian journal of ophthalmology 63.1 (2019): 15.

[213]- Hakim, Antoine, et al. "Gene therapy strategies for glaucoma from IOP reduction to retinal neuroprotection: progress towards non-viral systems." Advanced Drug Delivery Reviews (2023): 114781.

[214]- Chowdhury, Ekram Ahmed, et al. "Current progress and limitations of AAV mediated delivery of protein therapeutic genes and the importance of developing quantitative pharmacokinetic/pharmacodynamic (PK/PD) models." Advanced Drug Delivery Reviews 170 (2021): 214-237.

[215]- Deng, Yanhui, et al. "Age-related macular degeneration: Epidemiology, genetics, pathophysiology, diagnosis, and targeted therapy." Genes & diseases 9.1 (2022): 62-79.

[216]- Latifi-Navid, Hamid, et al. "Network analysis and the impact of Aflibercept on specific mediators of angiogenesis in HUVEC cells." Journal of Cellular and Molecular Medicine 25.17 (2021): 8285-8299.

[217]- Yu, Wenhan, and Zhijian Wu. "Ocular delivery of CRISPR/Cas genome editing components for treatment of eye diseases." Advanced drug delivery reviews 168 (2021): 181-195.

[218]- Torrecilla Alzola, Josune, et al. "MMP-9 Downregulation with Lipid Nanoparticles for Inhibiting Corneal Neovascularization by Gene Silencing." (2019).

[219]- Liu, Xiaochuan, et al. "SDF-1 Functionalized Hydrogel Microcarriers for skin flap repair." ACS Biomaterials Science & Engineering 8.8 (2022): 3576-3588.

[220]- Catala, Pere, et al. "Approaches for corneal endothelium regenerative medicine." Progress in retinal and eye research 87 (2022): 100987.

[221]- Mohanna, Seyedeh Zeinab Mirjalili, et al. "LNP-mediated delivery of CRISPR RNP for wide-spread in vivo genome editing in mouse cornea." Journal of Controlled Release 350 (2022): 401-413.

[222]- Fu, Dun Jack, et al. "Development of a corneal bioluminescence mouse for real-time in vivo evaluation of gene therapies." Translational Vision Science & Technology 9.13 (2020): 44-44.

[223]- Novelli, Flavia, et al. "p63 in corneal and epidermal differentiation." Biochemical and Biophysical Research Communications 610 (2022): 15-22.

[224]- Mohanna, Seyedeh Zeinab Mirjalili, et al. "LNP-mediated delivery of CRISPR RNP for wide-spread in vivo genome editing in mouse cornea." Journal of Controlled Release 350 (2022): 401-413.

[225]- Caruso, Salvatore Marco, et al. "CRISPR/Cas therapeutic strategies for autosomal dominant disorders." The Journal of Clinical Investigation 132.9 (2022).

[226]- Wilson, Mark R., et al. "Clusterin, other extracellular chaperones, and eye disease." Progress in Retinal and Eye Research 89 (2022): 101032.

[227]- Sarnicola, Caterina, Asim V. Farooq, and Kathryn Colby. "Fuchs endothelial corneal dystrophy: update on pathogenesis and future directions." Eye & contact lens 45.1 (2019): 1-10.

[228]- Rong, Ziye, et al. "Trinucleotide repeat-targeting dCas9 as a therapeutic strategy for Fuchs' endothelial corneal dystrophy." Translational Vision Science & Technology 9.9 (2020): 47-47.

[229]- Sawtell, Nancy M., and Richard L. Thompson. "Alphaherpesvirus latency and reactivation with a focus on herpes simplex virus." Current Issues in Molecular Biology 41.1 (2021): 267-356.

[230]- Faria-e-Sousa, Sidney Júlio, and Rosalia Antunes-Foschini. "Herpes simplex keratitis revisited." Arquivos Brasileiros de Oftalmologia 84 (2021): 506-512.

[231]- Abolhosseini, Mohammad, et al. "A triad of microscopes for rapid and proper diagnosis of infectious keratitis." Clinical and Experimental Optometry 105.3 (2022): 333-335.

[232]- Elbadawy, Hossein Mostafa, et al. "Targeting herpetic keratitis by gene therapy." Journal of ophthalmology 2012 (2012).

[233]- Faurez, Florence, et al. "Biosafety of DNA vaccines: New generation of DNA vectors and current knowledge on the fate of plasmids after injection." Vaccine 28.23 (2010): 3888-3895.

[234]- Zhang, Qing, and Fusheng Liu. "Advances and potential pitfalls of oncolytic viruses expressing immunomodulatory transgene therapy for malignant gliomas." Cell death & disease 11.6 (2020): 485.

[235]- Rathnasinghe, Raveen, et al. "Interferon mediated prophylactic protection against respiratory viruses conferred by a prototype live attenuated influenza virus vaccine lacking non-structural protein 1." Scientific Reports 11.1 (2021): 22164.

[236]- Jamali, Arsia, et al. "Characterization of resident corneal plasmacytoid dendritic cells and their pivotal role in herpes simplex keratitis." Cell reports 32.9 (2020).

[237]- Jamali, Arsia, et al. "Characterization of resident corneal plasmacytoid dendritic cells and their pivotal role in herpes simplex keratitis." Cell reports 32.9 (2020).

[238]- Lorentz, Holly, and Heather Sheardown. "Ocular delivery of biopharmaceuticals." Mucosal Delivery of Biopharmaceuticals: Biology, Challenges and Strategies (2014): 221-259.

[239]- Pace, Lanny W. "PRESENT POSITION AND ADDRESS." (2017).

[240]- Marino, Andreana, et al. "Role of herpes simplex envelope glycoprotein B and toll-like receptor 2 in ocular inflammation: an ex vivo organotypic rabbit corneal model." Viruses 11.9 (2019): 819.

[241]- Matundan, Harry, and Homayon Ghiasi. "Herpes simplex virus 1 ICP22 suppresses CD80 expression by murine dendritic cells." Journal of virology 93.3 (2019): e01803-18.

[242] Shoji, Jun, et al. "A diagnostic method for herpes simplex keratitis by simultaneous measurement of viral DNA and virus-specific secretory IgA in tears: an evaluation." Japanese journal of ophthalmology 60 (2016): 294-301.

[243]- Huang, Mengwen, et al. "Mucosal vaccine delivery: A focus on the breakthrough of specific barriers." Acta Pharmaceutica Sinica B (2022).

[245]- Dhanushkodi, Nisha Rajeswari, et al. "Mucosal CCL28 Chemokine Improves Protection against Genital Herpes through Mobilization of Antiviral Effector Memory CCR10+ CD44+ CD62L− CD8+ T Cells and Memory CCR10+ B220+ CD27+ B Cells into the Infected Vaginal Mucosa." The Journal of Immunology (2023).

[250]- Tian, Bo, et al. "Ocular drug delivery: advancements and Innovations." Pharmaceutics 14.9 (2022): 1931.

[246]- Sadeghian, Issa, et al. "Potential of cell-penetrating peptides (CPPs) in delivery of antiviral therapeutics and vaccines." European Journal of Pharmaceutical Sciences 169 (2022): 106094.

[247]- Zhu, Shuyong, and Abel Viejo-Borbolla. "Pathogenesis and virulence of herpes simplex virus." Virulence 12.1 (2021): 2670-2702.

[248]- Villa, Erica, et al. "Dynamic angiopoietin-2 assessment predicts survival and chronic course in hospitalized patients with COVID-19." Blood advances 5.3 (2021): 662-673.

[249]- Chapellier, Benoit, et al. "Meganuclease targeting HSV-1 protects against herpetic keratitis: Application to corneal transplants." Molecular Therapy-Nucleic Acids 30 (2022): 511-521.

[250]- Harrell, C. Randall, et al. "Therapeutic potential of mesenchymal stem cells and their secretome in the treatment of glaucoma." Stem cells international 2019 (2019).

[251]- Picaud, Serge, et al. "The primate model for understanding and restoring vision." Proceedings of the National Academy of Sciences 116.52 (2019): 26280-26287.

[252]- Becker, Jonas, Julia Fakhiri, and Dirk Grimm. "Fantastic AAV gene therapy vectors and how to find them—random diversification, rational design and machine learning." Pathogens 11.7 (2022): 756.

[253]- Sharif, Najam A. "Therapeutic drugs and devices for tackling ocular hypertension and glaucoma, and need for neuroprotection and cytoprotective therapies." Frontiers in pharmacology 12 (2021): 729249.

[254]- van Mechelen, Ralph JS, et al. "Animal models and drug candidates for use in glaucoma filtration surgery: A systematic review." Experimental eye research 217 (2022): 108972.

[255]- Rahić, Ognjenka, et al. "Novel drug delivery systems fighting glaucoma: Formulation obstacles and solutions." Pharmaceutics 13.1 (2020): 28.

[256]- Naik, Santoshi, et al. "Small interfering RNAs (siRNAs) based gene silencing strategies for the treatment of glaucoma: Recent advancements and future perspectives." Life Sciences 264 (2021): 118712.

[257]- Shen, Junhui, Yuanqi Wang, and Ke Yao. "Protection of retinal ganglion cells in glaucoma: Current status and future." Experimental Eye Research 205 (2021): 108506.

[258] Sun, Difang, et al. "Long-term and potent IOP-lowering effect of IκBα-siRNA in a nonhuman primate model of chronic ocular hypertension." Iscience 25.4 (2022): 104149.

[259]- Ng, Si Yun, and Alan Yiu Wah Lee. "Traumatic brain injuries: pathophysiology and potential therapeutic targets." Frontiers in cellular neuroscience 13 (2019): 528.

[260]-Tan, Junkai, et al. "C3 transferase-expressing scAAV2 transduces ocular anterior segment tissues and lowers intraocular pressure in mouse and monkey." Molecular Therapy-Methods & Clinical Development 17 (2020): 143-155.

[261]-Bannier-Hélaouët, Marie, et al. "Exploring the human lacrimal gland using organoids and single-cell sequencing." Cell stem cell 28.7 (2021): 1221-1232.

[262]-Molino, Jay. "Microcápsulas vacías empleadas en aplicaciones de ultrasonidos: generalidades, usos y métodos de fabricación." (2019).

[263]-Bastola, Prabhakar, et al. "Adeno-associated virus mediated gene therapy for corneal diseases." Pharmaceutics 12.8 (2020): 767.

[264]-Singh, Vimal Kishor, et al. "Current approaches for the regeneration and reconstruction of ocular surface in dry eye." Frontiers in Medicine 9 (2022): 885780.

[265]-Yao, Yuan, et al. "Immunobiology of T cells in Sjögren's syndrome." Clinical Reviews in Allergy & Immunology 60 (2021): 111-131.

[266]-Pflugfelder, Stephen C., and Michael E. Stern. "Biological functions of tear film." Experimental eye research 197 (2020): 108115.

[267]-Alam, Jehan, Cintia S. de Paiva, and Stephen C. Pflugfelder. "Immune-Goblet cell interaction in the conjunctiva." The ocular surface 18.2 (2020): 326-334.

[268]-Xu, Yining, et al. "Surface modification of lipid-based nanoparticles." ACS nano 16.5 (2022): 7168-7196.

[269]-Singh, Neera, et al. "Epithelial barrier dysfunction in ocular allergy." Allergy 77.5 (2022): 1360-1372.

[270]-Nagai, Noriaki, and Hiroko Otake. "Novel drug delivery systems for the management of dry eye." Advanced Drug Delivery Reviews (2022): 114582.

[271]-Shah, Ruchi, et al. "Systemic diseases and the cornea." Experimental eye research 204 (2021): 108455.

[272]-Hampe, Christiane S., et al. "Mucopolysaccharidosis type I: a review of the natural history and molecular pathology." Cells 9.8 (2020): 1838.

[273]-Loret, Amaury, et al. "Joint manifestations revealing inborn metabolic diseases in adults: a narrative review." Orphanet Journal of Rare Diseases 18.1 (2023): 1-11.

[274]-Salman, Mohd, et al. "New frontier in the management of corneal dystrophies: basics, development, and challenges in corneal gene therapy and gene editing." The Asia-Pacific Journal of Ophthalmology 11.4 (2022): 346-359.

[275]-Del Rio, Danila, et al. "CAV-2 vector development and gene transfer in the central and peripheral nervous systems." Frontiers in Molecular Neuroscience 12 (2019): 71.

[276]-D'Oria, F., R. Barraquer, and J. L. Alio. "Crystalline lens alterations in congenital aniridia." Archivos de la Sociedad Española de Oftalmología (English Edition) 96 (2021): 38-51.

[277]-Luboń, Wojciech, et al. "Understanding ocular findings and manifestations of systemic lupus erythematosus: Update review of the literature." International Journal of Molecular Sciences 23.20 (2022): 12264.

[278]-Daruich, Alejandra, et al. "Congenital aniridia beyond black eyes: from phenotype and novel genetic mechanisms to innovative therapeutic approaches." Progress in Retinal and Eye Research (2022): 101133.

[279]-Taha, Eman A., Joseph Lee, and Akitsu Hotta. "Delivery of CRISPR-Cas tools for in vivo genome editing therapy: Trends and challenges." Journal of Controlled Release 342 (2022): 345-361.

[280]-Latta, Lorenz, et al. "Pathophysiology of aniridia-associated keratopathy: Developmental aspects and unanswered questions." The Ocular Surface 22 (2021): 245-266.