Literature Review

Results and possible prospects of genetic technology in ophthalmology (literature review). Part I

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The emergence of fundamentally novel technological solutions in the field of gene therapy today, the formation of the priority and the development of genetic technologies create serious prerequisites for the beginning of a new Fusion era in ophthalmology in the near future. This review, in its first part, presents the results of fundamental and clinical studies on the use of viral and non-viral systems for the delivery of genetic material in ophthalmology. The second part of the review will focus on genetic therapeutic strategies (gene replacement, gene suppression, genomic editing using CRISPR / Cas9 technology, priming and transposon editing) that have been used in ophthalmology over the past several years.

The basic Principles of the Predictive, Preventive and Personalized Medicine as the Healthcare Model of the near future, as well as the Strategy for the Development of Medical Science in Russia for the Period until 2025, require that researches in healthcare (particularly, but not only, eye care) shall focus on the areas associated with the genetic, cellular, nano- and information technologies [1].

The emergence of fundamentally novel technological solutions in the field of gene therapy and gene diagnostics, the creation of priority for and development of genetic technologies create serious prerequisites for the beginning of a new Fusion era in ophthalmology in the near future.

Before we overview current genetic technologies in ophthalmology and make the analysis of global trends in these technologies, we consider current normative legal support and maintenance in the area of developing genetic technologies.

The Federal Research Program for Genetic Technologies Development until 2027 was approved in 2019 [2]. A Program's key objective is to implement a comprehensive solution to the task of the accelerated development of genetic technologies, including genetic editing.

The meeting of the Working Group on Legal Regulation in the Area of Genetic Technologies, Including Genetic Editing and Bioethics on October 16, 2020, adopted a decision on the establishment of the Center for Law and Bioethics Related to Genomic Research and Technologies, the principal aim of which is to provide legal support for the accelerated development and application of genetic technologies.

The Center for Precision Genetic Editing and Genetic Technologies in Biomedicine has been established within the framework of the program and the national project named "Science".

Gene therapy. Gene delivery systems

The gene delivery systems can be divided into two classes, viral and non-viral; either has its advantages and disadvantages.

Viral gene delivery systems

Recombinant-deficient viral vectors (viruses that are no longer able to replicate) are commonly used for the delivery of genetic material into cells. The types of vectors available for the delivery include adenoviruses, adeno-associated viruses, lentiviruses and herpes simplex viruses.

Adenoviruses have a rather high capacity for gene transfer (their vectors can carry long fragments of DNA, more than 20,000 base pairs, or 20 kb), a high level of transduction (DNA transfer to target cells), and their viral DNA does not integrate into the host cell DNA (the genetic material remains in the cytoplasm and is not delivered into the nucleus, which is both an advantage and a disadvantage, because transgene expression is transient); in addition, they are highly immunogenic (not safe) [3, 4].

Recombinant adeno-associated viruses (AAV) are attractive vectors for several reasons: they are non-pathogenic, have a low immunogenicity, display broad tropism to most cells and tissues, high transduction efficacy,

integrate into the host DNA at the particular site, and can trigger long-term transgene expression. One limitation of recombinant AAV is that their genome-packaging capacity is not ≤ ~ 5 kb.

Experimental studies on the use of AAV for induced neovascularization models of retinal and optic nerve disorders (age-related macular degeneration (AMD), pigmented retinitis, Leber's amaurosis and Stargardt disease) have been widely conducted in recent years [5-19]. Retinal, corneal, and trabecular meshwork transductions have been found to be effective for the various AAV variants and various routes of AAV delivery. Boye and colleagues [20] used a mixture of viscoelastic (Healon) and AAV to place sub-inner limiting membrane (ILM) injections in the primate retina, and demonstrated uniform and extensive transduction of retinal ganglion cells (RGCs) in the areas beneath the subILM bleb. Simpson and colleagues [21] suggested that AAV-PHP.eB, a novel AAV9-based mutant capsid, crosses the intra-retinal blood-retinal barrier (IR-BRB), efficiently transduces horizontal cells located adjacent to IR-BRB, but has very limited ability to further penetrate retina and reach photoreceptors. Lee and colleagues [22] evaluated the transduction efficiencies of four different AAV serotypes, AAV2, 5, 8, and 9, in streptozotocin (STZ)-induced diabetic mouse retinas. AAV2 and AAV9 displayed the most efficient transduction. Particularly, AAV2 was transduced into various retinal cells, including Müller cells, microglia, retinal ganglion cells (RGCs), bipolar cells, horizontal cells, and amacrine cells, whereas AAV9 was effectively transduced only into RGC and horizontal cells [22]. In a mouse study by Wang and colleagues [23], a synthetically developed AAV transduced following a single intracameral injection efficiently the trabecular meshwork, corneal stroma, and endothelial cells. In a mouse study by Lee and colleagues [24], laser photocoagulation induced transduction of retinal cells by intravitreally administered AAV.

Lentiviruses (LV) belong to the family of retroviruses, have a rather small genome-packaging capacity (≤8 kb), can trigger long-term transgene expression, and induce minimal host immune response. However, they randomly integrate into the genome and can cause insertional mutagenesis by disrupting other genes, leading to severe adverse events. In ophthalmological studies, lentiviruses have been less frequently used for genetic material delivery than AAV. They have been used for vitreoretinal disorders, AMD, diabetic retinopathy, choroidal melanoma, retinoblastoma, neovascularization, and corneal dystrophy [11, 25-29]. In an interesting rat study by Bai and colleagues [30], LV mediated toll-like receptor 2 (TLR2) small interference RNA (SiRNA) could effectively down regulate the TLR2 expression via RNA interference and prolong the survival of corneal grafts, although not necessarily able to prevent the rejection.

Therefore, currently, fundamental studies on the use of AAV and LV for the delivery of genetic material into the eye are widely conducted. Particularly, they found that: (1) retinal, corneal, and trabecular meshwork transductions are effective for the various AAV variants and various routes of AAV delivery; (2) different AAV serotypes differ in transduced cells and tissues of the eye; (3) the efficacy of genetic therapy mostly depends on the route of delivery.

As at August, 2019, according to online Wiley library records (http://www.abedia.com/wiley/indications.php), the total number of clinical studies on gene therapy drugs was 3000. Of these, 95% were phase 1/2 studies, and 5% were phase 2/3 studies. In addition, as at August, 2019, postmarketing studies were being conducted only for 5 gene therapy drugs. Only 9 gene therapy drugs have been approved for use by the Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA).

Luxturna (Spark Therapeutics, Philadelphia, PA), an AAV-based vector carrying a normal copy of the RPE65 gene to the RPE cells that are lacking a normally functioning RPE65 gene, was approved for the treatment of biallelic RPE65mutation–associated retinal dystrophy (Leber congenital amaurosis) by the FDA in 2017, and by the Committee for Medicinal Products for Human Use (CHMP) of the EMA in 2018. Luxturna is injected subretinally once per eye, for patients who have confirmed RPE65 mutations and enough viable cells in their retina [31, 32]. The first commercial gene therapy with Luxturna was performed at the Children's Hospital Los Angeles in 2018.

Efficacy and safety studies of AAV-mediated delivery of the genetic material to the eye have been widely conducted in recent years.

GenVec (Gaithersburg, MD) assessed the safety of a single intravitreous injection of an E1-, partial E3-, E4-deleted adenoviral vector expressing human pigment epithelium-derived factor (AdPEDF.11) for advanced neovascular AMD in a phase I clinical trial [33]. There were no serious adverse events related to AdPEDF.11, but signs of mild, transient intraocular inflammation occurred in 25% of patients.

Avalanche Biotechnologies (Menlo Park, CA) in collaboration with the Australian Lions Eye Institute investigated the safety profile and effectiveness of subretinal injections of rAAV sFLT-1 recombinant AAV (rAAV) soluble fms-like tyrosine kinase-1 (sFLT-1) for neovascular AMD in Phase 1 and 2a human clinical trials [34, 35]. A subretinal injection of a low-dose (1×1010 vector genomes (vg)) or high-dose (1×1011 vg) sFLT-1 was found to be safe, and administration of anti-VEGF gene therapy for neovascular AMD was found to be promising.

CD59 complement factor is a membrane-bound inhibitor of the membrane attack complex (MAC), an immune protein that mediates cell lysis by the formation of plasma membrane pores. Hemera Biosciences (Waltham, MA) carried out a phase I gene therapy trial to evaluate the safety of MAC inhibition via intravitreal delivery of an adeno-associated virus vector (AAV2) that expresses the soluble form of membrane-independent CD59 (sCD59),

AAVCAGsCD59 (HMR59), in participants with dry AMD [36]. The trial found HMR-59 to be safe and effective, and, of the patients with at least 6 months of therapy, 18% did not require anti-VEGF treatment within this period [37].

Oxford BioMedica (UK) Ltd carried out a phase I trial in patients with advanced NVAMD, the Gene Transfer of Endostatin/angiostatin for Macular Degeneration Trial (GEM Study), to test the safety and bioactivity of subretinal injection of a lentiviral Equine Infectious Anemia Virus (EIAV) vector expressing the angiogenesis inhibitors endostatin and angiostatin (RetinoStat®) [27]. The study findings demonstrated that lentiviral EIAV vectors provide a safe platform with robust and sustained transgene expression for ocular gene therapy.

Adverum Biotechnologies (Menlo Park, CA) started a phase I trial to study the safety profile of intravitreal ADVM-022, a novel recombinant AAV, in 2018. ADVM-022 utilizes the AAV2.7m8 capsid, which carries a strong, ubiquitous expression cassette encoding a codon-optimized cDNA of the aflibercept protein. Patients with AMD and active CNV had an intravitreal injection of 6E11 vg/eye or 2 6E11 vg/eye as per the study protocol. The study is still ongoing and the final results have not been presented as yet.

A phase 1/2 study evaluated the safety and tolerability of RGX-314 (RegenexBio) in patients with wet AMD. RGX-314 is an AAV8 vector encoding for a soluble anti-VEGF Fab protein, which binds to RPE cells to produce a therapeutic anti-VEGF protein. The gene encodes for an anti-VEGF fragment of a monoclonal antibody. The safety of different doses of RGX-314 (3E9 GC, 1E10 GC, 6E10 GC, 1.6E11 GC, and 2.5E11 GC) was evaluated in a phase 1 trial. Stabilization of the process was observed in 73% of the cases within 6 months, and in 50% of the cases within 18 months, and patients did not require anti-VEGF treatment within these periods. A long-term follow-up study of RGX-314 (RGX-314 LTFU) was initiated in December, 2019.

Non-viral gene delivery systems

These include plasmid DNA that is either delivered directly (termed “naked DNA”) or complexed with carriers (e.g., metal-based, polymer-based or lipid-based nanoparticles). Non-viral gene delivery systems may offer a less invasive, low immunogenic and inflammatory response than viral systems and can package rather large plasmids. What is more, and important to consider, they are more attractive for manufacturers because their manufacturing cost is lower and they are more easily standardized. The major disadvantage is low efficiency of transfection of the genetic material into a cell.

Naked DNA is the simplest gene delivery system. In this case, they use a gene which is joined by a relatively low number of nucleotide sequences. Although there have been relatively few reports on studies with naked DNA, they found the reporter plasmid DNA to be efficiently transduced and transfected into retinal cells [38, 39].

Metal-based, polymer-based and lipid-based nanoparticles are more common and successful gene delivery systems compared to naked DNA.

Metal-based (cerium oxide and yttrium oxide) nanoparticles are non-toxic for ocular tissues and provide antioxidant protection in retinal disorders [40-44]. Polymer-based nanoparticles have recently attracted the attention of researchers because they can be rather easily manufactured and have controllable characteristics. Particular attention is paid to the CK30-PEG compacted DNA nanoparticles that have been successfully tested in the eye. These particles have been demonstrated to be non-immunogenic in various tissues. In addition, in several studies, they have been used for gene transfer in models of retinitis pigmentosa and diabetic retinopathy, and were supposed to be promising for these disorders [45, 46]. Lipid-based delivery systems (liposomes, DNA-lipid complexes, and etc.) are currently one of the most commonly used types of nanoparticle carriers. Gao and colleagues [47] constructed the orongoxygen induced retinopathy (OIR) mouse (C57BL/6J) model and evaluated targeting VEGF siRNA transfection by new polymeric liposomes to inhibit retinal neovascularization. They found this approach to produce a durable therapeutic effect, with a significantly reduced area of retinal neovascularization [47]. A disadvantage of lipid-based delivery systems is the lack of cell specificity which may result in non-target effects. Wang and colleagues [48] demonstrated that cell-specific promoters enable lipid-based nanoparticles to deliver genes to specific cells of the retina in vivo.

A novel method of targeted delivery of the genetic material has been recently developed, which enables transfection of rather large gene constructs. The technique utilizes polymer-covered gold nanorods and near-infrared laser irradiation, with gold nanoparticles having a maximum absorption spectrum in this region. These nanorods are heated with application of a laser pulse, and, when nanoparticles are in contact with the cell membrane, the nanorods enable site-specific transfection of large gene constructs due to increased permeability of the cell membrane without non-target effects [49].

Conclusion

Therefore, the results of ophthalmological clinical studies demonstrate that applications of gene therapy drugs delivered by viral (mainly, AAV) and non-viral systems are safe and effective, and that gene therapy seems promising for the treatment of various eye disorders. We, however, need to bear in mind that (a) AAV can show immunogenicity (especially, associated with repeat injections), (b) LLV can cause insertional mutagenesis and carcinogenesis, and (c) liposome-based delivery systems do not always allow for cell-specific transport mechanisms, which can produce non-target effects. Today, the major objectives in the area of systems for gene therapy delivery to the eye are targeted delivery of the genetic material and transduction/transfection of large therapeutic genes.

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Table 1. Clinical trials of viral vector gene therapy drugs for ocular diseases

Drug	Mecha-nism of action	Deve-loper	Route	Clinical trial phase/ reg. number (Clinicaltrials.gov)	Ocular disease	Comments
AAV2-hRPE65v2 (Luxturna)	AAV	Spark Therapeutics	Subretinal	3 NCT00999609	Leber's amaurosis	Favorable safety profile FDA approved in 2017 EMA approved in 2018
AdGVPEDF.11D	AAV	GenVec	Intravitreal	1 NCT00109499	Neovascular AMD	There were no serious adverse events related to AdPEDF.11, but signs of mild, transient intraocular inflammation occurred in 25% of patients.
rAAV.sFLT-1	AAV	Avalanche Biotechnologies	Subretinal	1/2a NCT01494805	Neovascular AMD	Favorable safety profiles of a subretinal delivery of a low-dose (1×1010 vg) and high-dose (1×1011 vg) sFLT-1
RGX-314	LV	RegenexBio	Subretinal	1/2a NCT03066258	Neovascular AMD	Favorable safety profile Stabilization of the process was observed in 73% of the cases within 6 months, and in 50% of the cases within 18 months, and patients did not require anti-VEGF treatment within these periods. A long-term follow-up study of RGX-314 (RGX-314 LTFU6; NCT03999801) was initiated in December, 2019.
RetinoStat	LV	Oxford Biomedica	Subretinal	1 NCT01301443	Neovascular AMD	Favorable safety profile
AAVCAGsCD59 (HMR-59)	AAV	Hemera Biosciences	Intravitreal	1 NCT03585556	Neovascular AMD	Favorable safety profile Of the patients with at least 6 months of follow-up, 18% did not require anti-VEGF treatment within these 6 months.
ADVM-022	AAV	Adverum Biotechnologies	Intravitreal	1 NCT03748784	Neovascular AMD	The final results have not been presented as yet.

Notes: AAV, adeno-associated viral vector; LV, lentiviral vector; VG, gene vectors; AMD, age-related macular degeneration