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Retinal Gene Therapy: Current Progress and Future Prospects

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Abstract

Clinical trials treating inherited retinal dystrophy caused by *RPE65* mutations had put retinal gene therapy at the forefront of gene therapy. Both successes and limitations in these clinical trials have fueled developments in gene vectors, which continue to further advance the field. These novel gene vectors aim to more safely and efficiently transduce retinal cells, expand the gene packaging capacity of AAV, and utilize new strategies to correct the varying mechanisms of dysfunction found with inherited retinal dystrophies. With recent clinical trials and numerous pre-clinical studies utilizing these novel vectors, the future of ocular gene therapy continues to hold vast potential.

Keywords

gene therapy; inherited retinal dystrophies; RPE65; adeno-associated virus; equine infectious anemia virus

Inherited retinal dystrophies (IRD) encompass a diverse group of devastating blinding disorders affecting approximately 1 in 3000 people [1]. Of these rare disorders, retinitis pigmentosa (RP) is the most common and associated with defects in over 60 genes. Altogether, RP and other IRDs involve mutations in over 200 genes, with greatly varying mechanisms of visual dysfunction (RetNet, http://www.sph.uth.tmc.edu). To add to the complexity of these disorders, the aforementioned mutations can result in autosomal recessive, autosomal dominant, x-linked, and even digenic inheritance. These diverse mechanisms of disease pose a unique challenge for gene therapy. For example, autosomal recessive mutations necessitate gene replacement. Autosomal dominant mutations, which can result in dominant negative effects or toxic gain of function, might require gene suppression with or without subsequent gene replacement. Some genes are too large for current vectors. For these diseases where direct gene replacement is not practical, expression of neuroprotective factors that act in a mutation independent fashion might be needed. Finally, conventional gene replacement therapy would not be beneficial in patients with

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severe or complete loss of photoreceptors. The application of gene therapy to express light-sensitive proteins in inner retinal cells has given rise to the field of optogenetics. Gene therapy techniques continue to advance and evolve to overcome these challenges in new and innovative approaches.

Leber congenital amaurosis (LCA), a more severe dystrophy associated with defects in at least 21 genes was the first ophthalmological disease targeted with gene therapy. Clinical trials of gene replacement therapy for LCA due to *RPE65* (retinal pigmented epithelium-specific protein 65kDa) mutations (*RPE65*-LCA) [2–4] have highlighted the immense potential for treating IRDs, especially those caused by loss of function mutations. Among early viral vector prototypes, such as adenovirus, lentivirus, and adeno-associated virus (AAV); AAV showed the best combination of safety and retinal cell transduction [5]. Following the success of *RPE65*-LCA trials, AAV has emerged as the leading delivery vector for ocular gene therapy. However, many challenges and questions remain as to how to broaden the spectrum of treatable diseases as well as how to better regulate and sustain gene expression. Although AAV2 has been shown to efficiently transduce RPE cells, newer vectors are needed to selectively and efficiently target other retinal cells, particularly photoreceptors. Second, the 4.7 kb gene capacity of AAV has hindered treatment of mutations in larger genes. Developments in AAV dual vectors and non-AAV viral vectors have opened up gene targets such as *CEP290*, *ABCA4*, and *MYO7A*, associated with LCA, Stargardt, and Type 1B Usher syndromes. However, some genes, such as *USH2A*, still exceed the capacity of these newer techniques and will likely require alternative techniques, such as compacted DNA nanoparticles [6].

We will briefly review the seminal *RPE65*-LCA studies with a focus on how findings and limitations of these studies have shaped the future direction of ocular gene therapy. *RPE65*-LCA studies have highlighted potential adverse effects from a subretinal delivery, which have spurred advancements in viral gene vectors and attempts to achieve efficient photoreceptor cell transduction through a safer intravitreal delivery route [7]. This review will cover advancements in gene therapy that have since emerged and broadened treatment possibilities, and the new frontiers in treatment of ocular and inherited retinal diseases being explored.

RPE65-LCA clinical trials: how we got there and what have we learned

The RPE65 story provides an ideal example of how translational research can move from the clinic to the lab bench top and ultimately back to the clinic. While genetic testing might be able to identify the causative mutation, much preclinical work was necessary to produce a treatment. Elucidation of the function of RPE65 at the cellular level permitted an understanding of the mechanism of the disease. The identification and creation of both small and large animal models furthered this understanding and provided a platform for the development of treatments. Finally, an understanding of the disease phenotype provided the evidence that human clinical trials would be feasible.

In LCA, the most severe of retinal dystrophies, patients show nystagmus, unrecordable electroretinograms (ERGs), and a profound visual deficiency at birth or within the first few

years of life that progresses to total blindness by mid- to late-adulthood. Cross sectional genotype-phenotype studies have suggested that *RPE65*-LCA patients show a slower and less severe photoreceptor degeneration relative to other LCA genes. For example, patients at the earliest ages tested have been found to have severely reduced but detectable cone electroretinograms [8,9], greater visual acuity within the first decade of life [10–12], recordable visual fields with peripheral concentric constriction [10–12], and residual islands of retained vision in adulthood [9,12]. Another characteristic phenotype of *RPE65*-LCA patients is the relative preservation of the outer nuclear layer (ONL) demonstrated through *in vivo* structural optical coherence tomography (OCT) studies, which was in agreement with ONL preservation in animal models. An advanced modeling study further demonstrated that ONL preservation in patients was greater than expected for the level of vision loss in the fovea and in rod-rich regions temporally and superiorly to the fovea [13]. This greatly encouraged advancements into gene therapy since successful visual gains depended on the presence of surviving photoreceptors.

The initial step in understanding *RPE65* was the identification and cloning of the *RPE65* gene in humans and mice [14], followed closely by the generation of the *RPE65* knockout mouse [15]. Comprehensive pre-clinical characterization of multiple mouse (*rd12*, $Rpe65^{R91W}$-knockin, and *Rpe65*-knockout mice) and canine (*RPE65*-mutant Briard dog) models of *RPE65*-LCA had elucidated important groundwork information on the pathophysiology of disease. RPE65 is an isomerase in the retinoid cycle involved in regeneration of the essential visual pigment, 11-*cis*-retinal, specifically in conversion of all-*trans*-retinyl esters to 11-*cis*-retinol [15]. With loss of function of RPE65, there is progressive retinal degeneration [15], a decrease in lipofuscin granules, and accumulation of retinyl esters in lipid droplets in RPE cells [15–17]. A lack of 11-*cis*-retinal regeneration leads to a severe decrease in visual function with abnormal rod- and cone-mediated electroretinograms (ERGs) at approximately 10% of normal levels by 2–4 months of age in *Rpe65*-KO mice [18]. Despite visual function deficits, histologic studies showed relatively slow rod photoreceptor loss in all animal models [18]. *Rpe65*-KO mice show a particularly severe cone cell loss following early cone opsin misclocalization, in contrast to the foveal sparing in *RPE65* patients. Foveal preservation may stem from an inherent resistance to degeneration, residual enzymatic activity with specific RPE65 mutations, and an alternative isomerase in Muller glial cells for chromophore regeneration [19]. While gene replacement would not restore vision loss secondary to photoreceptor degeneration, it could increase the 11-*cis*-retinal chromophore to increase photon capture and subsequent light sensitivity.

In addition to the promising characteristics of the *RPE65* phenotype, simultaneous developments in AAV vectors were essential to the road towards treatment of *RPE65*-LCA. Adeno-associated virus (AAV) is a linear single-stranded DNA virus belonging to the *Parvoviridae* family and the *Dependovirus* genus, first found as a contaminant amongst adenovirus (Ad) [20]. The high safety profile, transduction efficiency and longevity of AAV over other viruses have established it as a leading vector [20]. AAV vectors largely exist as an episomal, non-integrating virus [21], which ensures stable transgene expression in postmitotic cells. Wildtype AAV shows non-pathogenicity in humans with 40–80% of adults seropositive without associated symptoms or disease [22]. Despite its safety,

neutralizing antibodies have been reported to decrease vector efficiency, particularly with systemic administration [23]. Although less likely to be problematic in ocular tissues, a potential reduction in transduction efficiency is concerning in administration of rAAV treatment to the contralateral eye.

The prototypic AAV2 used in *RPE65*-LCA clinical trials, was first cloned into a bacterial plasmid in 1982 [24,25]. Currently, production of recombinant AAV vectors involves co-transfection of two engineered plasmids. The first plasmid is the transgene cassette that has removed all viral genes except for two palindromic inverted terminal repeats (ITRs) flanking the transgene of interest [26,27]. This replication-deficient plasmid carrying the transgene of interest requires co-transfection with plasmids expressing viral replication (*rep*) and packaging capsid (*cap*) genes (Figure 1).

With well-defined animal models and advancements in AAV gene delivery, the next progression towards treatment for *RPE65*-LCA patients was proof-of-concept vision rescue in animal models. Subretinal administration of AAV2 packaging the *Rpe65* gene under a hybrid cytomegalovirus (CMV) immediate early enhancer/chicken β-actin (CBA) promoter provided vision rescue as measured through ERGs in 1–2 month old *Rpe65*-KO and *rd12* mice [28]. The same AAV2 construct successfully provided long-term improvements up to 3 years in the canine *RPE65*-mutant model in rod- and cone-ERGs, and 11-*cis*-retinal regeneration [29]. A variety of AAV pseudotypes and gene promoters in studies from other groups also showed visual improvements in the *RPE65*-canine model [30–32], as well as long-term improvements in photoreceptor outer segment morphology and lipid inclusions [31]. Although pre-clinical canine studies showed high safety profiles, a potential adverse effect of dose-dependent retinal thinning with AAV2-CBA-*hRPE65* has been observed [33].

Initiation of three independent *RPE65*-LCA clinical trials in 2007 occurred following the mounting evidence of safe and successful proof-of-concept visual improvements across multiple *Rpe65* animal models and the promising phenotype of *RPE65*-LCA patients. All studies demonstrated a relatively high safety profile of AAV2-*RPE65* gene replacement without toxicity, adverse surgical events, or serious immunologic events [2–4]. Slight differences in visual outcomes have been reported between studies, particularly as long-term follow-ups are released. Changes in visual acuity (VA), which is often the gold standard outcome for clinical trials, have been variable. This is not surprising considering the low baseline VA in many of the treated patients. In initial patient cohorts, one of three trials reported VA improvements in three out of three patients [4], while insignificant changes in VA were found in the other two trials. However, the patients with improved VA showed the lowest baseline VA, with improved VA generally not surpassing the baseline VA of the other patients from the initial cohorts. In addition to individual baseline visual function, differences in visual outcomes may be attributed to the differences between the three independent clinical trials in AAV viral vector production, gene promoter use, and dosage concentration and volume of viral solution administered (Table 1). No direct comparisons of efficiency have been conducted on these three specific gene promoters used. In addition to varying volume, subtle differences in administration methods may alter the amount of solution forming the subretinal bleb. Despite these differences, a larger number of patients

from each trial showed improved visual sensitivity as assessed through microperimetry [2], full-field sensitivity testing [3], and transient pupillary light response (TPLR) [4] (Table 2).

A particularly interesting finding from one group of investigators has been the development of “pseudo-foveas” initially observed in one patient at one year post-treatment [34], with similar findings in an additional three patients in further follow-up studies [7,35]. These patients demonstrated the worst baseline VAs with non-foveal fixation [7]. They gained the greatest improvements in VA post-treatment, which was found to stem from improved vision at their extrafoveal fixation locus or “pseudo-fovea”, an area of best visual function.

Cautionary tales from early trials using first-generation AAV vectors

Despite encouraging visual gains in the Phase I studies, three out of five *RPE65*-LCA patients in one trial had significant foveal thinning at 1 or 3 year post-operative follow-up [7]. While one patients’ thinning was attributed to the natural course of the disease, the cause of thinning in the other two patients was less apparent. Unfortunately, foveal thinning was not measured in other clinical trials, limiting a full assessment of the risks and benefits of this treatment. It remains unclear whether foveal thinning in patients was a complication secondary to vector toxicity, foveal detachment following subretinal injection, or a combination of these factors. Elucidation of factors that might contribute to potential photoreceptor loss will be important in developing the next generation of vectors. Erring on the side of caution, it has been therefore suggested to include the fovea only when there is already significant foveal atrophy and when fixation is parafoveal [7]. However, treatment of the fovea will be essential to prevent significant vision loss as younger patients are treated and in other diseases such as Stargardt dystrophy.

Overcoming obstacles to improve efficacy

Since development of the first-generation rAAV2 vector, vast innovations and improvements in viral gene vectors have emerged largely driven by inefficient transduction of immune-competent organ systems, such as the liver, muscle, and heart [36]. These advancements address the potential causes of foveal thinning in *RPE65* patients in vector toxicity, transgene overexpression, and adverse events from subfoveal detachment.

Optimizing viral transduction with AAV vector advancements

In canine models, cone cell loss occurred in a dose-dependent manner indicating the potential for cellular toxicity due to high viral titers [33]. Therefore, one strategy to prevent potential vector toxicity is to lower the number of viral particles used. Viral transduction has been enhanced through various approaches, such as providing improved cell-specific tropism to decrease off-target transduction, increased transduction efficiency, and through the evasion of immune responses that would otherwise decrease efficacy. Although distinctly enumerated below, alterations to the viral capsid may achieve more than one of these advantageous properties simultaneously or synergistically.

Improvements in cell-specific tropism

One innovation in AAV capsid engineering has been the development of pseudotyping. Following the characterization of AAV2, AAV serotypes 1 through 9 were subsequently isolated (reviewed in [37]) and it became evident that differences in capsid proteins directed cell tropism. Taking advantage of variations in tropism, “pseudotyped” rAAV vectors express AAV2 replication proteins with capsid proteins of other serotypes (Figure 1). In the retina, all serotypes thus far have shown RPE cell transduction, with AAV1, 2, 4, and 6 transducing RPE cells either specifically or more efficiently than neural retina [38–41]. Conversely, retinal photoreceptor transduction is best achieved with AAV5, 7, 8, and 9 [40–42]. Selecting the appropriate AAV pseudotype would effectively reduce the viral titer required for transduction of the desired retinal cell type.

Improved transduction efficiency through directed mutagenesis

Beyond pseudotyping, AAV capsid engineering through directed capsid peptide mutagenesis has improved transduction efficiency by minimizing viral vector degradation. In directed mutagenesis, specific surface-exposed tyrosine residues are altered to phenylalanine (Y-F mutants) to evade tyrosine phosphorylation, which would normally target the vector for ubiquitination and proteasome-mediated degradation [43] [44]. Therefore, these capsid mutants escape degradation and show improved intracellular trafficking and transduction efficiency [44,45]. In addition to improved efficacy, Y-F mutants showed novel tropism, with some Y-F mutants achieving photoreceptor- and RPE-specific cell targeting, whereas others provided widespread pan-retinal transduction [45,46].

Surface-exposed tyrosine residue mutants also mitigate binding of neutralizing antibodies to the neutralizing epitope regions in the AAV capsid [46], that would otherwise prevent efficient viral binding and uptake into desired cells [47]. This potential adverse immune response may prohibit effective viral re-administration and/or treatment of the contralateral eye.

Improved transduction efficiency through directed evolution

In contrast to site-directed mutagenesis, AAV capsid engineering via directed evolution introduces random large-scale alterations to the capsid envelop. While various strategies of directed evolution have been thoroughly reviewed [48–50], the general methodology first involves generation of a large library of chimeric AAV capsid mutants through combinatorial techniques such as error prone PCR that generate random mutagenesis, site specific mutagenesis at key binding sites, and DNA shuffling of multiple *cap* genes (Figure 2). AAV capsid libraries are subsequently applied to selection conditions in *in vitro* or *in vivo* systems to generate desired phenotypes, such as the aforementioned retinal properties. AAV variants that are able to effectively transduce specific cells, bind to receptors, or overcome barriers or antibodies are identified and selected for amongst the large library of AAV capsid mutants.

Improved transgene expression through gene promoters

Strategic use of gene promoters can also impart cell-specific tropism and improved transduction efficiency, providing another means of viral transduction optimization thus reducing the number of viral particles needed. While ubiquitous promoters, such as CMV and CBA have classically been used to obtain maximal expression across multiple cell types, there is a movement toward achieving cell-specific physiological levels of expression. Compared to the wide therapeutic range of *RPE65* replacement, gene replacement of other RPE genes such as *BEST1* [51] and *MERTK* [52] have utilized the retinal pigmented epithelium-specific vitelliform macular dystrophy 2 (VMD2) promoter.

Photoreceptor-specific genes are particularly fastidious and might need to be well-titrated to achieve sufficient but non-toxic levels of expression, such as with *RHO* [53–55], *MYO7A* [56], or *BBS1* [57]. Cone cell damage with overexpression of the reporter *GFP* gene has also been reported [58]. In these cases, photoreceptor-specific opsin and human G-protein-coupled receptor protein kinase 1 (hGRK1) promoters have been utilized [59]. A number of promoters have also been developed for cone-specific targeting, including the human red opsin pR2.1 promoter [60], a 569 bp S-opsin promoter known as HB569 [61], and an chimeric IRBPe-GNAT2 (inter-retinoid binding protein enhancer element – transducin alpha-subunit) promoter [62], largely useful for treatment of achromatopsia (ACHM).

Minimizing cell loss through better surgical methods

In addition to vector toxicity, foveal thinning in *RPE65* patients may have occurred secondary to subfoveal detachment. To avoid this potential adverse outcome, novel methods of creating a subretinal bleb have been developed [63] and alternate intravitreal routes of photoreceptor transduction have been highly sought after [64].

The delicate nature of the degenerating retina poses inherent surgical challenges and requires extreme care with subretinal administration of gene vectors. A recent two-step injection technique attempts to minimize trauma from generation of the subretinal bleb. An initial subretinal detachment is formed over the targeted area through injecting a balanced salt solution applied through a continuous footswitch-operated pressure device. The viral vector is subsequently deposited through the same retinotomy site, extending the existing detachment site [63].

Directed evolution of viral vectors has opened up the possibility of using intravitreal rather than subretinal injections [64]. While Y-F capsid mutants provided initial proof-of-concept for intravitreal transduction of outer retinal cells in mice [46,65], transduction was highly limited or absent in canine [66] and non-human primates (NHPs) [64]. This discrepancy has been partly attributed to the biophysical barrier imparted by the significantly thicker inner limiting membrane (ILM) in large animals [67]. Additionally, a complex balance occurs between a vector's ability to bind to ILM proteins such as heparan sulfate and laminin. For example, AAV2, AAV8 and AAV9 bind heparan sulfate, while AAV1 and AAV5 primarily bind sialic acid, a monosaccharide that is absent in the ILM [68]. The "7m8" capsid mutant, produced through directed evolution, contains a seven-amino acid loop insertion in the heparan sulfate binding domain [64]. It has demonstrated the most effective effective pan-

retinal transduction via intravitreal delivery in NHPs to date, improving upon the results from Y-F mutants. Greater retinal penetration of the 7m8 clone in NHPs was likely attributed to reduced heparan sulfate affinity which decreased sequestration at the ILM.

Expansion of gene therapy: large genes and new disease frontiers

Following the success of *RPE65* clinical trials, a multitude of gene targets are currently or will soon be entering clinical trials (Table 3 and Table 4). However, the endogenous 4.7 kb packaging capacity of AAV has limited utility in treating larger genes. Particularly desirable large gene targets are *ABCA4* and *MYO7A*, mutations which cause Stargardt (STGD1) and Usher (USH1B) syndromes. Expanding the packaging capacity is therefore an important second aim in AAV vector development. Multiple strategies are currently being explored including: oversized AAV vectors, dual AAV vector systems, non-AAV viral vectors such as equine infectious anemia virus (EIAV), and compacted DNA nanoparticles.

"Overstuffed" AAV vectors

With oversized or "overstuffed" AAV vectors, transgenes exceeding the conventional 4.7 kb capacity are packaged as usual. A sizeable extension to a packaging capacity of 8.9 kb was reported specifically with AAV5, with other overstuffed AAV serotypes showing a significant decrease in viral titer production [69]. Although this study showed that oversized AAV5 viral particles held intact, full-length genomes that mediated *in vitro* and *in vivo* full-length expression [69], further studies have had more limited success. Although additional studies produced oversized AAV1–5 and AAV8 vectors packaging 5.2 kb [70] to 6.0 kb [71], it was noted that viral titer yields may be decreased ten-fold compared to AAV < 4.7 kb [70] and oversized vectors may undergo intracellular proteasomal degradation post-entry [71]. In contrast to successful encapsulation of full-length genomes [69,71], other studies demonstrated fragmentation of genomes greater than 5.3 kb that subsequently recombine intracellularly to produce full-length expression [70,72]. These discrepancies between studies in the ability to encapsulate oversized genomes without fragmentation, size capacity, and transduction efficiency are controversial and remains to be further examined.

Dual AAV vectors

In contrast to a single oversized vector, three strategies to accommodate larger genes utilize a dual vector approach where the transgene of interest is divided into 5' and 3' halves (Figure 3). The first strategy, known as overlapping vectors, overtly takes advantage of recombination between two halves of the transgene that contain a homologous overlapping sequence [73]. The second strategy termed, trans-splicing, utilizes vectors containing a 3' splice donor signal on one cassette and a 5' splice acceptor signal on a second cassette, with head-to-tail concatemerization of the two vectors producing full-length transgene expression [74]. A third hybrid strategy adds a highly recombinant region from an exogenous gene, such as alkaline phosphatase (AP) and the F1 phage (AK), and places it adjacent to the splice sites, thus utilizing both recombination and splicing [75,76].

Use of dual vector AAV approaches in retinal applications has been hindered by several factors. Dual vector systems require co-transfection of two vectors in a single cell with

subsequent occurrence of homologous recombination or splicing of gene products. The low levels of homologous recombination that occurs in post-mitotic neurons, such as photoreceptors, decreases the efficiency of overlapping vectors significantly [76]. Additionally, *in vivo* retinal transduction efficiencies of trans-splicing and hybrid dual vectors have been highly variable, ranging from ∼5% to 100% of the transduction efficiency of single AAV vectors [76,77], although the reason for this variability remains unknown. Lastly, while no aberrantly truncated proteins were detected *in vivo*, thorough evaluation of this would be required moving forward into clinical trials.

Non-AAV viral vectors for retinal gene therapy

With these limitations in extended capacity of AAV vectors, alternative non-AAV vectors with large packaging capacities have been examined. One particular viral vector that has shown promise is the equine infectious anemia virus (EIAV). EIAV is a non-HIV1-based, non-primate lentivirus in the *Retroviridae* family able to package transgenes up to 8 kb [78]. Although it is associated with a self-limiting equine infectious anemia in horses, donkeys, and mules; humans show no pre-existing immunity or pathogenicity to EIAV [79]. It has shown a high safety profile in CNS, muscle, and hematopoietic pre-clinical studies [80–82]. In fact, EIAV was the first lentivirus used in clinical trials for the treatment of Parkinson's disease [83], following its demonstrated safety and efficacy in simultaneously delivering three enzymes involved in dopamine synthesis to rodent [84] and NHP [85] disease models. In ocular studies, EIAV has shown high safety and transduction efficiency in neonatal rodent [56,86,87] and adult NHP photoreceptors [79,88].

The use of HIV1-based lentiviral vectors has been more limited than non-HIV1-based lentiviruses in retinal gene therapy due to inefficient photoreceptor transduction in adult retina [41,89]. No HIV1 pseudotypes have been found to significantly improve photoreceptor transduction in adult rodents to date [78,90]. While similar findings in rodents were also reported with EIAV, EIAV efficiently transduces photoreceptors in adult NHPs, whereas photoreceptor transduction with an HIV1-lentiviral vector in *ex vivo* adult human retinal explants remained highly inefficient [91]. Definitive *in vivo* NHP studies with HIV1 vectors are required. The utility of HIV1-based lentivirus, however, may be in RPE transduction with HIV-1-Mokola [41] and HIV1-venezuelan equine encephalitis virus-derived glycoprotein (VEEV-G) [91] pseudotypes showing improved RPE transduction. Another limitation in HIV1-lentiviral vectors stems from its property of random integration into the chromosome. Recent developments in integration-deficient vectors, however, may address concerns of insertional mutagenesis [92].

Although adenoviral vectors offer a large packaging capacity of up to 37 kb [93], the transient transgene expression following adenoviral vector gene delivery had limited its use. This decrease in transgene expression is attributed to immunogenic responses that lead to clearance of the virus and/or of transduced cells [94]. The development of helper-dependent adenoviral vectors, which are devoid of all viral genes except for encapsidation, has improved long-term transgene expression following subretinal delivery [90]. Additionally, improved photoreceptor targeting through alterations to the penton base in combination with

use of photoreceptor-specific gene promoters [95,96] has recently increased the utility of adenoviral vectors in the treatment of IRDs.

Nanoparticles as an alternative vector to deliver large genes

A last technology developed to overcome size limitations with AAV vectors has been DNA-compacted nanoparticles (NPs), which are single DNA molecules compacted polyethylene glycol-substituted polylysine 30-mers (CK30PEG). Its 20 kb packaging capacity [97] provides immense possibilities in gene targets with particular utility in treatment of Type 2A Usher syndrome associated with mutations in the *Usherin* gene, where its 15.6 kb size prohibits packaging into any AAV or non-AAV viral vectors. Its safety and utility has been demonstrated in the lung [98], with introduction of the cystic fibrosis transmembrane conductance regulator (CFTR) through an intranasal route resulting in Phase I/II clinical trials for cystic fibrosis patients [99]. In retinal gene therapy, nanoparticles have also demonstrated safety and transduction efficiency in *Abca4*-KO mice [6]. However, nanoparticles have shown some limitations hindering its widespread use in ocular gene therapy. It showed lower transduction efficiency per vector genome as compared to AAV [100], and silencing of transgene expression potentially related to the gene promoter [100] or the DNA plasmid backbone used [101], thus higher vector titers and repeated dosing might be required. Although not problematic in intranasal treatment of lung diseases, repeated subretinal injections would be less than ideal.

Other retinal gene therapies underway or in preparation

Ocular gene therapy has extended to a whole host of eye conditions, including expansion of the gene target size, treatment of autosomal dominant and X-linked IRDs, and expression of anti-angiogenic genes to treat neovascular age-related macular degeneration (Table 3 and Table 4). Treatment has also gone beyond simple gene replacement into gene-independent strategies, such as expression of neuroprotective agents and light-sensitive proteins with optogenetics.

Gene therapy for Stargardt dystrophy

Mutations in the 6.8 kb *ABCA4* gene are associated with STGD1, which is the most common form of juvenile-onset macular degeneration. *ABCA4* encodes for ABCR, a flippase for *N*-retinylidene phosphatidylethanolamine (*N*-retinylidene-PE) to the cytoplasmic side of photoreceptor outer segment disk membranes. A deficiency of the protein leads to accumulation of all-*trans*-retinal, *N*-retinylidene-PE and the subsequent toxic by-product, *N*-retinylidene-*N*-retinylethanolamine, commonly known as A2E [102]. Successful treatment of the commonly used *Abca4*-STGD1 mouse model has been demonstrated with a variety of these large gene capacity vectors, including the oversized AAV5 vector [69], the trans-splicing and hybrid AAV2 dual vector systems [76], and the EIAV vector [86]. However, only EIAV has thus far has been translated into clinical trials with recent initiation of escalating dose Phase I/IIa EIAV-*ABCA4* StarGen^{tm} clinical trials (Sanofi), following its demonstrated safety in NHPs [88].

Gene therapy for Usher Type 1B

The second important large gene target was the 6.6 kb *MYO7A* gene associated with Type 1B Usher syndrome, the most common inherited combined deaf-blind condition [103]. A spectrum in the severity of deafness and retinal degeneration defines the phenotypes of Usher 1, 2, and 3. Usher 1 patients show the greatest severity in hearing and visual impairment, with progression of retinitis pigmentosa beginning in childhood and profound deafness from birth to within a year of life. *Shaker1* (*sh1*) mice, lacking the *Myo7a* gene, are deaf and exhibit a characteristic circling and head shaking behavior for which it is named after due to cochlear and vestibular dysfunction. In the retina, myosin VIIa is an actin-based motor protein involved in rhodopsin transport in photoreceptor cilia, melanosome localization at RPE microvilli, and phagosome motility within RPE cells. *Sh1* mice therefore display ultrastructural opsin and melanosome mislocalization defects. Gene replacement in *sh1* mice with the oversized AAV5 vector [69], dual vector AAV2 [76], and the EIAV vector [56,87] successfully rescued ultrastructural defects. Similar to *ABCA4*, safety studies in NHPs [87] allowed for the EIAV-*MYO7A* vector, known as UshStat^{tm}, to enter Phase I/IIa dose escalation clinical trials (Sanofi).

Gene therapy for treatment of age-related macular degeneration

While neovascular age-related macular degeneration (AMD) has been successfully treated with routine repeated intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF) compounds, gene therapy alternatively could provide stable expression of anti-angiogenic molecules for a prolonged period. The large packaging capacity of EIAV has been used to treat neovascular AMD with RetinoStat^{tm}, an EIAV vector expressing two anti-angiogenic genes, endostatin and angiostatin. Subretinal delivery of RetinoStat^{tm} provided safe and efficient transgene expression in rodents [104] and NHPs [105], and significantly suppressed choroidal neovascular lesions [104]. These proof-of-concept studies progressed RetinoStat^{tm} into Phase I clinical trials (Oxford Biomedica) (Table 3), with potential to also improve ocular neovascularization in multiple conditions, such as diabetic retinopathy [106,107], retinopathy of prematurity (ROP) [108,109], and post-transplant neovascularization [110].

Successful treatment for neovascular AMD through gene therapy has also been achieved through intravitreal delivery of AAV2 expressing soluble Flt1 receptor (sFlt01), a chimeric VEGF binding agent [111]. Following the demonstrated safety, long-term expression up to 18 months post-treatment, and efficacy in inhibiting choroidal neovascularization in NHPs [112,113], a dose-escalation Phase 1 study was initiated (Genzyme) (Table 3). NHP studies, however, pointed to several potential obstacles and precautions with intravitreal AAV2-sFlt01. The first was variable expression levels, attributed to unpredictable release and diffusion of the AAV vector in the vitreous. Additionally, mild to moderate inflammatory reactions against the AAV2 capsid were demonstrated in 4 out of 6 macaques receiving the highest viral titer. Although transient mild to moderate inflammation was found with EIAV-RetinoStat^{tm}, inflammation was found to be relatively persistent lasting 5 to 15 months post-treatment with intravitreal AAV2-sFlt01, which was also attributed to the slow diffusion and trapping of the vector in the vitreous [112].

Gene therapy treatment for X-linked retinoschisis

The only gene currently associated with XLRS is *RS1*, which encodes for retinoschisin (Rs1). Rs1 is a 224 amino acid secreted protein that binds anionic phospholipids on photoreceptor and bipolar cell membranes, potentially mediating cell-matrix and cell-cell interactions, and playing a role in maintaining photoreceptor inner segment stability, synaptic structure, and overall retinal architecture [114,115]. Defects in *RS1* lead to characteristic splitting or schisis of retinal layers and loss of ERG b-waves due to the disruption of synaptic processes. In X-linked retinoschisis (XLRS), retinal detachments are not uncommon and schisis cavities render the retina highly susceptible to further retinal damage with subretinal gene delivery. Therefore intravitreal delivery is desirable. Intravitreal gene replacement of *RS1* with AAV8 is currently in Phase I/IIa dose-escalation clinical trials (National Eye Institute), with high safety and efficacy demonstrated in *rs1*-deficient mice and in rabbits [116,117]. However, use of intravitreally injected AAV2 is also being explored (AGTC), following the long-term safety and efficacy of AAV2 demonstrated in *rs1*-knockout mice [118].

Gene therapy treatment for choroideremia

Subretinally injected AAV2 is being used in clinical trials for choroideremia (CHM). CHM is an X-linked recessive disease with characteristic fundus findings of peripheral retinal, choroidal, and RPE degeneration with macular-sparing until late in the disease progression. CHM is associated with functionally null mutations in the 1.9 kb *CHM* gene, encoding for Rab escort protein-1 (REP-1). REP-1 is involved in the post-translational prenylation lipid-modification of Rab small GTpases (Rabs) needed for proper intracellular vesicular transport [119]. Initial results have been promising from Phase I/II clinical trials from six patients treated with the initial dose of AAV2-*REP1* [63]. Two of the six patients showed improvements in visual acuity, and five out of the six patients showed improved retinal sensitivity as assessed through microperimetry [63].

Gene therapy for retinitis pigmentosa

Mutations in *MERTK*, which encodes for a transmembrane receptor of tyrosine kinases involved in RPE cell phagocytosis of photoreceptor outer segments, are associated with autosomal recessive retinitis pigmentosa and LCA with characteristic findings of discrete autofluorescent dots and subretinal debris [120,121]. A subset of patients display preservation of peripheral fields [120,121] showing potential for rescue. The well-characterized *Mertk*-RCS (Royal College of Surgeon's) rat model was previously successfully treated with AAV2 [122,123], with further optimization through use of the RPE-specific promoter, VMD2 [52]. This improved AAV2-hVMD2-*MERTK* vector, is currently being used in Phase I clinical trials targeting *MERTK*-RP patients (King Khaled Eye Hospital) (Table 3).

Gene therapy for GUC2YD-LCA

Many more pre-clinical trials are currently underway and show great promise for future entry into clinical trials (Table 4). One of the most common causes of LCA are mutations in *GUCY2D* (LCA1), accounting for about 20% of cases. Human *GUCY2D* and the mouse

Gucy2e isoform encode for the retinal guanylate cyclase-1 (GC1), an essential photoreceptor outer segment protein required for synthesis of the cGMP visual transduction signaling molecule. Similar to *RPE65* patients, *GUCY2D*–LCA1 patients were found to have relatively preserved macular ONL and retinal laminar architecture [124,125] despite severe vision loss. Combined with successful pre-clinical rescue of GC1-knockout mice [126] and double GC1- and GC2-knockout mice [127], *GUCY2D*–LCA therefore shows high potential for entry into clinical trials.

Gene therapy for CEP290-LCA

A last genotype that has been associated with advantageous macular ONL preservation is *CEP290*-LCA10 patients [124,128]. The focus on rescue of the preserved foveal cones in patients has been recapitulated through generation of an all-cone mouse model lacking *Cep290* [129,130], allowing for future testing of treatment efficacy. With recent characterization of the feline *Cep290* model [131] that also allows for pre-clinical large animal model testing and recent developments in large gene capacity vectors, the potential to treat mutations in the 6.4 kb *CEP290* gene is high.

Gene therapy for achromatopsia

In line with attempts to treat conditions with macular preservation and advancements in cone-cell targeting [61,62,132], the treatment potential of ACHM patients has been closely examined. This has been facilitated through structural imaging of the macula and cone cells through high-resolution spectral-domain OCT (sdOCT) [133,134] and adaptive optics in ACHM patients [135]. Although cross-sectional studies have shown variability and a lack of clear age-related disease progression [133,134], the pre-clinical successes of treatment of the canine *CNGB3*-ACHM model [136], the rodent *CNGA3*-ACHM model [137,138], and recent characterization of a large-animal sheep model [139] provide encouragement for potential future clinical trials.

Gene therapy for X-linked retinitis pigmentosa

In addition to autosomal recessive IRDs, X-linked and autosomal dominant IRDs are being targeted for treatment. *RPGR*, a ciliary protein that is associated with over 70% of cases of X-linked retinitis pigmentosa (XLRP) is an important gene target. Multiple rodent models of *RPGR*-XLRP exist with the *Rpgr*-knockout mouse, transgenic mutant *Rpgr* mouse models, as well as the naturally occurring *rd9* mouse model [140]. Rescue of two naturally occurring canine *RPGR* models [141] with AAV2/5-*hIRBP*-*hRPGR* has provided evidence for safety and efficacy.

Gene therapy for autosomal dominant retinitis pigmentosa

Autosomal dominant IRDs, such as autosomal dominant RP (adRP), provide more of a treatment challenge than gene replacement for autosomal recessive IRDs. The most common cause of adRP is mutations in *RHO*, encoding for the essential visual transduction rhodopsin protein. With the high heterogeneity of *RHO*-adRP mutations of over 150 different mutations (RetNet, http://www.sph.uth.tmc.edu), an efficient rescue strategy has been through RNA-interference (RNAi) with simultaneous introduction of a modified rhodopsin

gene replacement resistant to RNAi suppression. This two-vector strategy has demonstrated efficient rescue of the *RHO*-adRP *P347S RHO*-mutant mouse model [142].

Gene-independent strategies: neuroprotection and optogenetics

Distinct from gene replacement, gene therapy has extended to mutation-independent strategies that provide a generalized means of vision rescue without the need to identify genetic defects causing disease. The first strategy has been in expression of neuroprotective factors, which provide gene-independent rescue effects for retinal dystrophies. A particularly promising neuroprotective factor is rod-derived cone viability factor (RdCVF), a thioredoxin-like protein secreted by rods that promotes cone photoreceptor survival [143]. While previous subretinal delivery of the RdCVF provided some retinal protection in the *P23H–RHO* adRP mouse [144], gene expression of RdCVF provides an improved strategy. Intravitreal expression of RdCVF with the novel 7m8 AAV2 capsid mutant developed through directed evolutionary methods was found to improve degeneration in the *rd10* RP mouse model [145]. Administration of neuroprotective agents would primarily be useful in slowing down retinal degeneration while awaiting genetic characterization.

A second mutation-independent strategy is in the field of optogenetics. In addition to being gene-independent, the advantage of this strategy is to restore visual function following loss of photoreceptor cells. While thoroughly reviewed elsewhere [146,147], optogenetics briefly works through expression of light-sensitive proteins in residual inner retinal cells, such as retinal ganglion cells or bipolar cells. Expression of channelrhodopsin-2 (ChR2) and halorhodopsin, ionotropic rhodopsins found in green algae and halobacteria, respond to light stimulation through conformational changes that cause opening of the channel and cell depolarization [146]. Although visual-evoked potentials were demonstrated following expression of ChR2 in ganglion cells of the *rd1* mouse model of retinal degeneration [148,149], limitations include requirements for a high level of light stimulus and/or transgene expression [149]. Although a highly promising tool in gene therapy, much work remains in improvement of methodology, such as increasing sensitivity and generating ON and OFF light responses, and determining whether the inner retinal circuitry in various retinal degenerations would benefit from optogenetic conversion.

Expert commentary

Generally considered as the most compelling success story in gene therapy, the big picture achievements of *RPE65* clinical trials have been pivotal to the larger field of gene therapy. The seminal *RPE65*-LCA clinical trials have provided the foundation for translational success and demonstrated safety of AAV in ocular gene therapy. Equally astounding have been the rapid advancements in response to the issues that have surfaced from and since *RPE65* studies. These recent advances in viral vectors have diversely expanded ocular gene therapy to provide viable treatments for a vast array of inherited retinal degenerations and ocular diseases. With further developments on the horizon, the future prospects of ocular gene therapy are tremendous.

Despite these large-scale achievements, limitations in vision rescue exist with treatment of a severe *RPE65*-LCA phenotype. Identification of diseases and genotype-phenotype patient

subsets that may be more amenable to vision rescue is a key aspect in moving forward with ocular gene therapy. Continuing advancements in imaging, such as with high-resolution optical coherence tomography and adaptive optics, are needed to provide a more detailed genotype-phenotype characterization and identify individual patient candidates. Also needed are continuing technologies in measurements of visual function to standardize baseline and post-treatment visual outcomes in patients with inherited retinal degenerations.

With multiple divergent avenues currently being explored in further advancements to AAV, alternate use of EIAV, and use of non-viral nanoparticles, much work remains to demonstrate the same safety and efficacy of AAV2 in a clinical setting. However, AAV2-*RPE65* studies had provided an exemplary groundwork to base future pre-clinical and clinical trials on.

Five-year review

In the coming years, additional follow-up results from Phase I/IIa and initial results from Phase III *RPE65*-LCA clinical trials will provide important information on dosing, and the treatment efficacy and visual gains that can be realistically provided by gene replacement therapy for a severe LCA retinal degeneration phenotype. Preliminary results of the safety and efficacy of the EIAV vector in treatment of Stargardt and Type 1B Usher syndrome will also emerge, which may potentially shape the use of AAV or non-AAV viral vectors. However, rapid developments in AAV vectors continue to address and improve upon limitations with the first-generation AAV2 vector. The great diversity in AAV capsid modifications as well as the potential to expand the AAV packaging capacity continues to make AAV an adaptable vector with vast potential in treating multiple IRDs. Ongoing pre-clinical treatment of IRDs with these novel AAVs will soon elucidate the successful modifications that best improve transduction efficiency, retinal-cell specificity, and intravitreal transduction of outer retinal cells across the new diseases and genes being targeted, which will further influence movement into clinical trials.

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Key issues

1. Inherited retinal dystrophies (IRDs) are genetically heterogenous and show diverse mechanisms of disease and inheritance, such as autosomal dominant, X-linked, and digenic inheritance requiring more complex treatment than gene replacement therapy for autosomal recessive diseases. Novel gene therapy strategies are required to address these additional and more complex IRDs.
2. The translational pathway exemplified by *RPE65*-LCA studies included a thorough understanding of the mechanism of dysfunction in animal models, pre-clinical treatment in these animal models, and detailed characterization of patient phenotype. This pathway greatly contributed to the success of *RPE65* clinical trials and would serve well to be modeled in the treatment of additional IRDs.
3. Despite promising improvements in retinal sensitivity, foveal thinning occurred following subfoveal delivery of AAV2 in Phase I/IIa *RPE65*-LCA clinical trials in three patients. The cause of foveal thinning remains unclear, and may be attributed to the location of subretinal administration or viral vector toxicity.
    a. Assessment of risks and benefits of foveal inclusion are important in future studies.
    b. An alternate intravitreal route of foveal transduction is in development.
    c. Optimizing transduction efficiency may minimize viral vector toxicity.
4. The limited 4.7 kb packaging capacity of adeno-associated virus requires alternative strategies to target large gene mutations causing IRDs, including expansion of the AAV capacity, use of other viral vectors, and non-viral vectors.
5. Current clinical trials are using AAV2 to treat autosomal recessive RP associated with *MERTK* mutations, and X-linked recessive choroideremia associated with *CHM* mutations.
6. Current clinical trials are treating large gene targets *ABCA4* and *MYO7A* associated with Stargardt dystrophy and Type 1B Usher syndrome with EIAV, a non-AAV viral vector.
7. Application of gene therapy forstable expression of anti-VEGF compounds in the treatment of AMD.
8. Mutation-independent gene therapy strategies may be required to aid vision rescue.

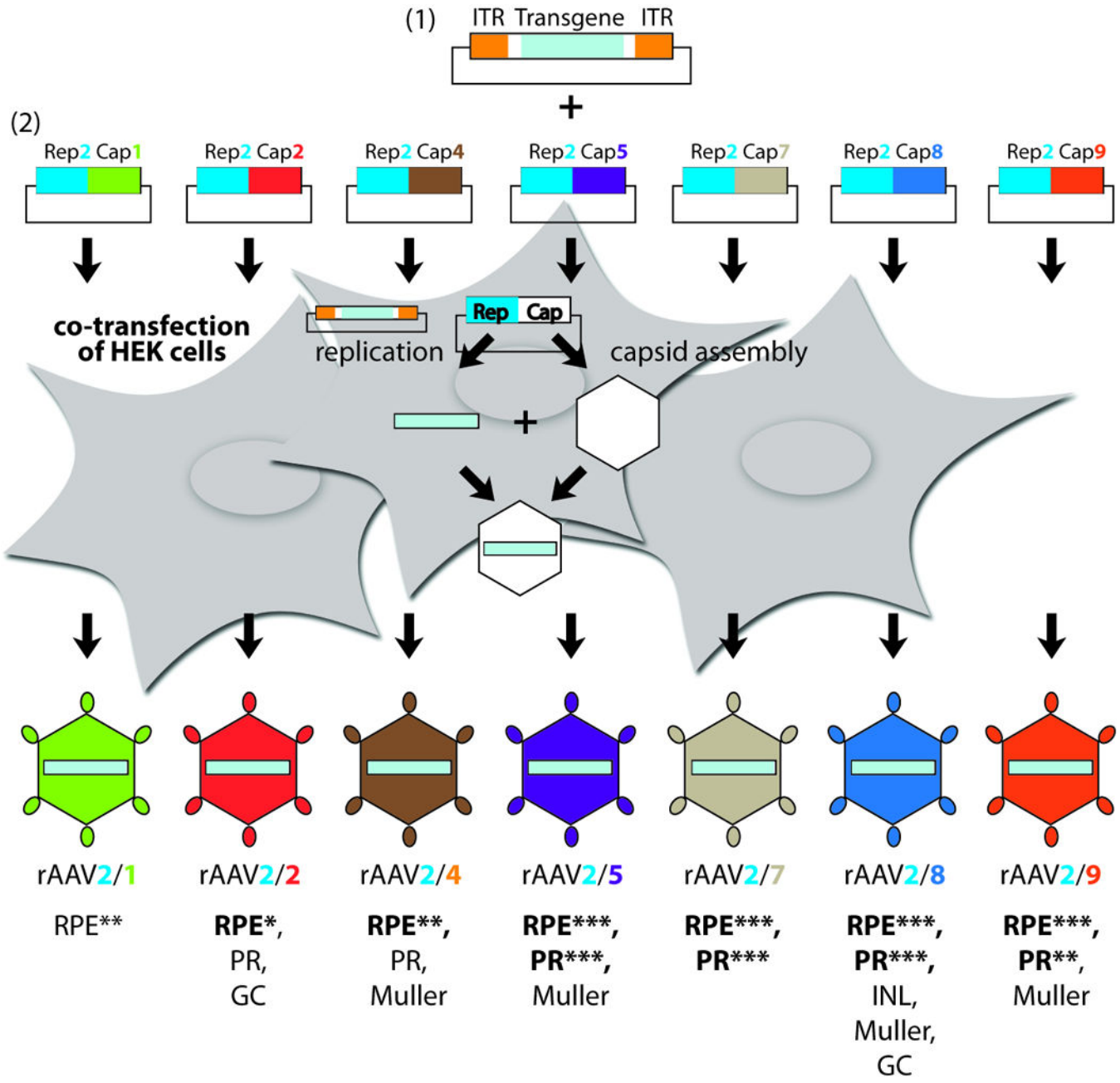

Figure 1.

AAV capsid pseudotyping and retinal cell tropism. Production of rAAV involves co-transfection of two plasmids: (1) the transgene cassette with two ITR regions flanking the transgene of choice, and (2) a helper plasmid encoding for the necessary replication (*Rep*) genes from AAV2 and capsid (*Cap*) genes from the desired AAV serotype. The retinal cell tropism of each AAV serotype listed below show the most effective retinal cell type transduced in *bold*. Relative comparisons of transduction efficiency of the RPE and PRs are shown from lowest (*) to highest (***) for particularly effective serotypes [38,42]. Note that information pertaining to transduction efficiencies is representative of murine retina [38,42],

but variances between species have been reported [150]. Abbreviations: ITR, inverted terminal repeats; rAAV, recombinant adeno-associated virus; RPE, retinal pigmented epithelium; PR, photoreceptors; GC, ganglion cells; INL, inner nuclear layer. Adapted from [39].

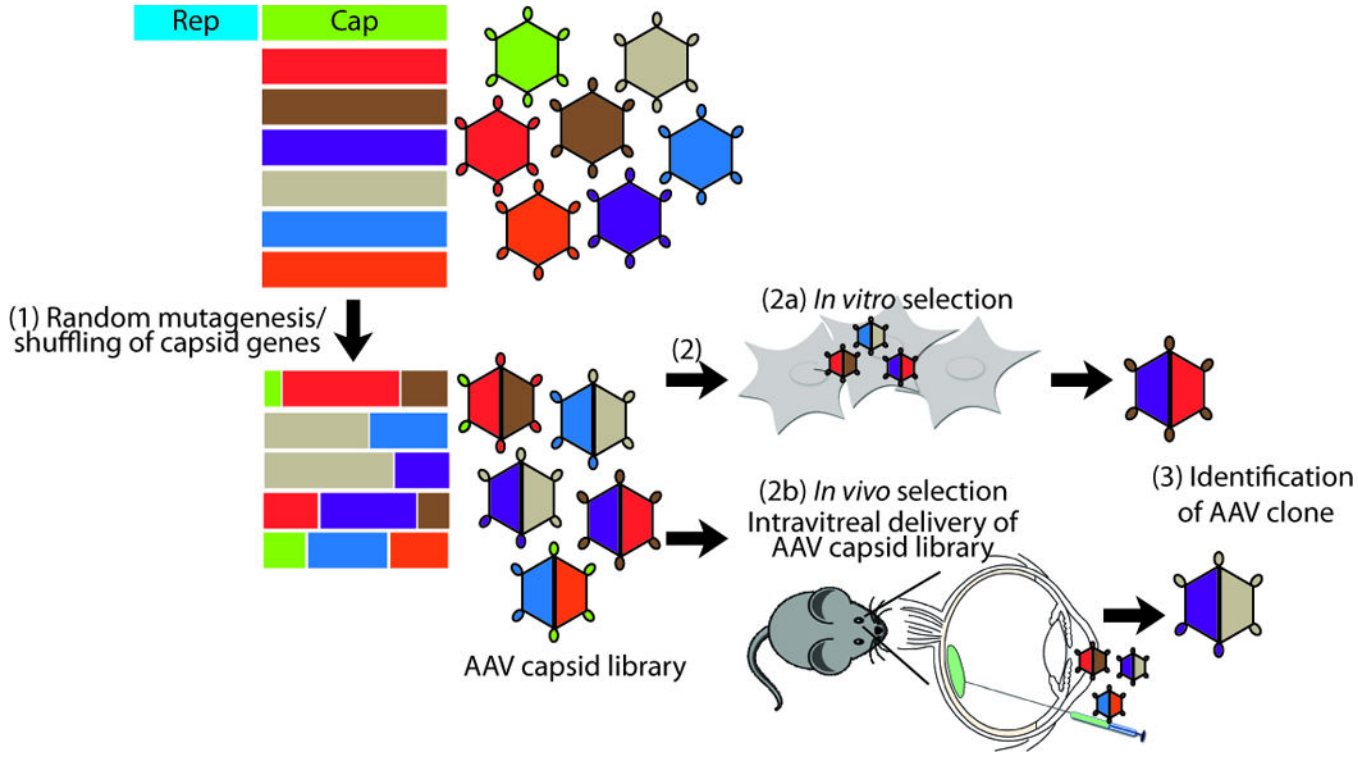

Figure 2.

AAV capsid engineering through directed evolution. Directed evolution involves (1) random mutagenesis, recombination, and/or DNA shuffling of AAV capsid genes from all AAV serotypes generate a large AAV capsid library with chimeric AAV capsids. (2) This capsid library is applied to (2a) *in vitro* or (2b) *in vivo* selection systems, and (3) AAV capsids that successfully overcome the selection criterion are identified.

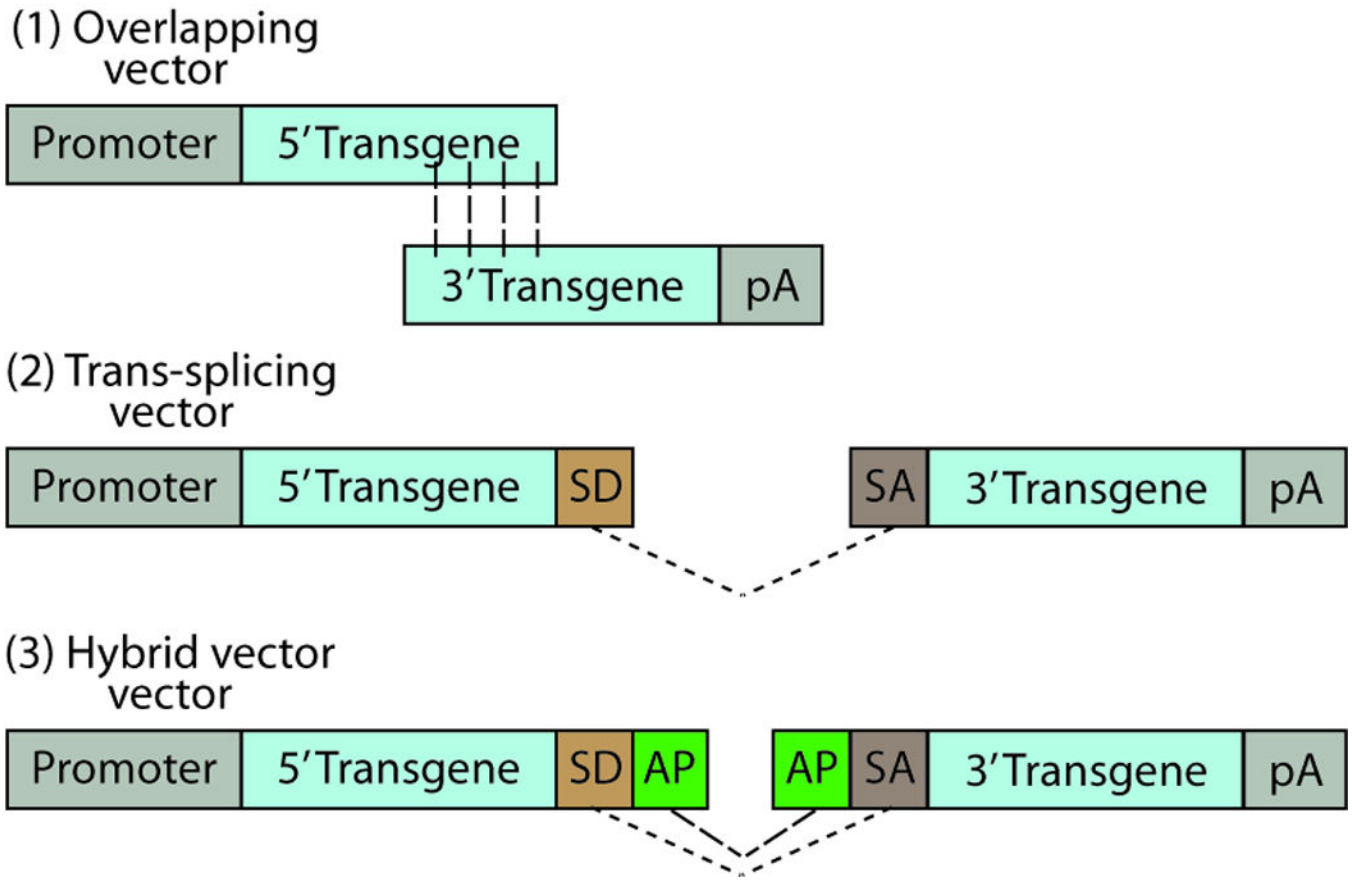

Figure 3.

Three AAV dual vector types. (1) Overlapping vectors have a homologous overlapping region between the two halves of the transgene of choice, which undergo homologous recombination for reconstitution of a full-length transcript of the transgene. (2) Trans-splicing vectors undergo splicing through their splice donor (SD) and splice acceptor (SA) sites at the ends of the transgene halves. (3) Hybrid vectors undergo splicing through the SD and SA sites, as well as recombination through a highly recombinogenic site from a exogenous gene, such as alkaline phosphatase (AP). Dashed lines show homologous recombination, dotted lines show splicing between SD and SA sites. Abbreviations: pA, polyadenylation tail; SD, splice donor; SA, splice acceptor; AP, alkaline phosphatase. Adapted from [76].

Table 1

***RPE65*-LCA Clinical Trials**

Details of *RPE65*-LCA clinical trials in gene promoter, viral titer, and volume of the viral solution used. *Results not yet published. **All clinical trial information was obtained from the Clinical Trials online registry, https://clinicaltrials.gov [165].

NCT#	Sponsor	Phase	Vector	Promoter	Viral titer (by cohort)	Volume (by cohort)	Subjects	Ref **[165]
00643747	UCL	I/II	rAAV2/2	hRPE65	1.00×10^{11} vp/ml	1.0 ml	N=12	[2]
00481546	U Penn - Scheie	I	rAAV2/2	CB^{SB}	1. 5.96×10^{10} vp/ml 2. 11.92×10^{10} vp/ml 3. 8. 94×10^{10} vp/ml 4. 17.88×10^{10} vp/ml 5. 17.88×10^{10} vp/ml (7.95×10^{10} vp/ml)	0.15 ml 0.30 ml 0.225 ml 2×0.225 ml 2×0.225 ml (0.20 ml)	N=3 N=3 N=3 N=2 N=4 (N=1)	[3,7,34,35]
00516477	U Penn - CHOP	I	rAAV2/2	CBA	1. 1.50×10^{10} vp/ml 2. 4.8×10^{10} vp/ml 3. 1.50×10^{11} vp/ml	0.15 ml 0.15 ml 0.30 ml	N=3 N=6 N=3	[4,151–155]
00749957	AGTC	I/II	rAAV2/2	CBA	1. 4.00×10^{11} vp/ml 2. 1.33×10^{12} vp/ml	0.45 ml 0.45 ml	N=6 N=6	[156]
00821340	Hadassah	I	AAV2/2	CB^{SB}	1.19×10^{11} vp/ml	0.30 ml	N=10	[157]
01496040	Nantes	I/II	rAAV2/4	hRPE65	6.0×10^{10} vp/ml	0.4–0.8 ml	N=9	[158]
00999609	Sparks Therapeutics	III	AAV2/2	CBA	1.50×10^{11} vp/mL	0.30 ml?	N=24	*

Abbreviations: NCT#, ClinicalTrials.gov identifier number; UCL, University College of London; U Penn-Scheie, University of Pennsylvania – Scheie Scheie Eye Institute; U Penn-CHOP, University of Pennsylvania – The Children's Hospital of Philadelphia. hRPE65p, human RPE65 promoter; hRPE65, human RPE65; CB^{SB}, hybrid modified short cytomegalovirus (CMV) enhancer and chicken β-actin promoter (CBA); vp, viral particles; ml, milliliter. Ref, references.

Table 2

Results from *RPE65*-LCA Clinical Trials

Visual outcomes of *RPE65*-LCA patients following treatment. *NCT#01208389 is a Phase I follow-up study treating the contralateral eye to patients enrolled in NCT#00516477. **All clinical trial information was obtained from the Clinical Trials online registry, https://clinicaltrials.gov [165].

NCT#	Sponsor	Cohort	Subjects	Reported improvements in visual outcomes						Ref **[165]
				VA	MP	FST	TPLR	Motility Studies	New or additional pseudofovea formation	
00643747	UCL	1	N=3	0/3 patients	1/3 patients	n/a	n/a	1/3 patients	n/a	[2]
00481546	U Penn - Scheie	1	N=3	0/3 patients	n/a	3/3 patients	2/3 patients	2/3 patients	1/3 patients	[3,7,34,35]
		2	N=3	1/3 patients	n/a	3/3 patients	3/3 patients	0/3 patients	2/3 patients	[7,35]
		3	N=3	1/3 patients	n/a	3/3 patients	3/3 patients	1/3 patients	0/3 patients	[7]
		4	N=2	1/2 patients	n/a	2/2 patients	1/2 patients	1/2 patients	0/2 patients	[7]
		5	N=4	0/4 patients	n/a	4/4 patients	2/4 patients	1/4 patients	1/4 patients	[7,35]
00516477	U Penn - CHOP	1	N=3	3/3 patients	n/a	3/3 patients	3/3 patients	3/3 patients	n/a	[4,151]
		2	N=6	3/6 patients	n/a	4/5 patients (1 n/a)	4/5 patients (1 n/a)	3/5 patients	n/a	[151]
		3	N=3	1/3 patients	n/a	1/2 patients (1 n/a)	3/3 patients	1/3 patients	n/a	[151]
*01208389	Spark Therapeutics	1	N=3 (N=12)	3/3 patients	n/a	2/3 patients	3/3 patients	2/3 patients	n/a	[154]

Abbreviations: NCT#, ClinicalTrials.gov identifier number; UCL, University College London; U Penn – Scheie, University of Pennsylvania, Scheie Eye Institute; U Penn – CHOP, University of Pennsylvania – The Children's Hospital of Philadelphia. VA, visual acuity; MP, microperimetry; FST, full-field sensitivity testing; TPLR, transient pupillary light response sensitivity; n/a, non-applicable or not-tested. Ref, references.

Table 3

Other Gene Therapy Trials under progress

Gene therapy clinical trials recently initiated. **All clinical trial information was obtained from the Clinical Trials online registry, https://clinicaltrials.gov [165]. Additional references in parentheses indicate pre-clinical data, and references without parenthesis indicate clinical trial publications.

NCT#	Trial status	Sponsor	Phase	Disease	Gene	Vector	Titer	Volume	Subjects	Ref **[165]
01367444	Recruiting	Sanofi	I/II	Stargardt	*ABCA4*	EIAV	n.p.	n.p.	N=8 N=4 N=4 N=12	([88])
01505062	Recruiting	Sanofi	I/II	Usher 1B	*MYO7A*	EIAV	n.p.	n.p.	N=6 N=3 N=3 N=	([56,87])
01461213	Recruiting	U Oxford	I II	CHM	*CHM*	AAV2	1×10^{10} vp/ml 1×10^{11} vp/ml	0.1 ml	N=6 N=6	[63]
02341807	Recruiting	Spark Therapeutics	I II	CHM	*CHM*	AAV2	n.p. n.p.	n.p. n.p.	N=5 N=5	([159,160])
02077361	Not yet recruiting	Ian M. MacDonald	I	CHM	*CHM*	AAV2	1×10^{10} vp/ml	0.1 ml	N=12	([159,160])
01482195	Recruiting	King Khaled Eye Hospital	I	LCA/RP	*MERTK*	AAV2	n.p.	n.p.	N=6	([52])
01301443	Active, not recruiting	Oxford Biomedica	I	AMD		EIAV	n.p. n.p. n.p. MTD	n.p.	N=3 N=3 N=3 N=9	([105])
01024998	Active, not recruiting	Genzyme/ Sanofi	I	AMD	*sFLT01*	AAV2	2×10^{8} vp/ml 2×10^{9} vp/ml 6×10^{9} vp/ml 2×10^{10} vp/ml MTD	0.1 ml 0.1 ml 0.1 ml 0.1 ml	N=6 N=6 N=6 N=6 N=10	([112])
01494805	Active, not recruiting	Lions Eye Institute/ Avalanche	I II	AMD	*sFLT01*	AAV	1×10^{10} vp/ml 1×10^{11} vp/ml	n.p.	N=40	([112])
02317887	Recruiting	NEI	I/IIa	XLRS	*RS1*	AAV8	1×10^{9} vp/ml 1×10^{10} vp/ml 1×10^{11} vp/ml	n.p.	N=3 N=3 N=3	([116,117])

Abbreviations: NCT#, ClinicalTrials.gov identifier number; U Oxford, University of Oxford; NEI, National Eye Institute. CHM, choroideremia; LCA, Leber congenital amaurosis; RP, retinitis pigmentosa; AMD, age-related macular degeneration; XLRS, X-linked retinoschisis. EIAV, equine infectious anemia virus; AAV, adeno-associated virus. vp, viral particles; ml, milliliter; n.p., not published; MTD, most tolerated dose. Ref, references.

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Table 4

Proposed Future Clinical Trials

Proposed or potential clinical trials following evidence of pre-clinical treatment efficacy.

Sponsor	Disease	Gene	Proposed Vector	Preclinical Evidence/ Animal Models	Ref	Sponser Ref
AGTC	Achromatopsia	*CNGB3*	AAV	Mouse (*Cngb3*-KO) Canine (*CNGB3*-KO, $CNGB3^{m/m}$)	[136,161]	[166]
AGTC	Achromatopsia	*CNGA3*	AAV	Mice (*Cpfl5Cnga3*-KO)	[137,138]	[166]
Genable	adRP	*RHO*	AAV	Mouse (*P347SRHO*)	[142]	[167]
University of Alberta	Choroideremia	*CHM*	AAV2	Mouse ($Chm^{null/WT}$)	[159,160]	[168]
Spark Therapeutics	Choroideremia	*CHM*	AAV2	Mouse ($Chm^{null/WT}$)	[159,160]	[169]
Genzyme	LCA Type 1	*GUCY2D*	AAV	Mice (*Gucy2e*–KO, *Gucy2e/Gucy2f* dKO)	[126,127,162]	[170]
	LCA Type 10	*CEP290*	AAV?	Mouse (*rd16; Nrl*−/−) Feline (*rdAc* Abyssinian cat)	[130,131]	
AGTC	XLRP	*RPGR*	AAV5	Canine (XLPRA1, XLPRA2)	[141]	[171]
AGTC	XLRS	*RS1*	AAV5	Mouse (*Rs1*-KO)	[118,163,164]	[172]
		RdCVF		Mouse (*rd10*)	[145]	

Abbreviations: AGTC, Applied Genetic Technologies Corp. adRP, autosomal dominant retinitis pigmentosa; LCA, Leber congenital amaurosis; XLRP, X-linked retinitis pigmentosa; XLRS, X-linked retinoschisis. *CNGB3*, cyclic nucleotide-gated channel beta 3; *CNGA3*, cyclic nucleotide-gated channel alpha 3; *RHO*, rhodopsin; *CHM*, choroideremia (Rab escort protein-1); *GUCY2D*, guanylate cyclase 2D, membrane (retina-specific), *CEP290*, centrosomal protein 290 kDa; *RPGR*, retinitis pigmentosa GTPase regulator; *RS1*, retinoschisin-1; RdCVF, rod-derived cone viability factor. AAV, adeno-associated virus; hIRBP, human interstitial retinol binding protein 3. KO, knockout; dKO, double knockout. Ref, references.

Preclinical evidence/animal models: $CNGB3^{m/m}$ canine model, misssense mutation in exon 6; *cpfl5* mouse, naturally occurring missense mutation in *Cnga3*; *P347S–RHO* mouse, missense mutation in *RHO* and a model of *RHO*-adRP; $Chm^{null/WT}$ mouse, female heterozygous-null carriers with the choroideremia phenotype; *Gucy2e*, guanylate cyclase 2E, membrane (retina-specific), the mouse homolog of *GUCY2D* or RetGC1, *Gucy2f*, guanylate cyclase 2F, membrane (retina-specific), the mouse homolog of *GUCY2F* or RetGC2; *Gucy2e/Gucy2f* dKO mice are missing both RetGC1 and RetGC2. *Rd16* mice, naturally occurring deletion of exons 35–39 of *Cep290*; *Nrl/-* mice, mouse lacking the neural retina leucine zipper gene involved in rod photoreceptor differentiation leading to an all-cone phenotype; *rdAC*, retinal degeneration Abyssinian cat with truncation mutation in *CEP290*; XLPRA1, X-linked progressive retinal atrophy-1, canine with ORF15 microdeletion (del 1028–1032) in *RPGR*; XLPRA2, X-linked progressive retinal atrophy-2, canine with ORF15 microdeletion (del 1084–1085) in *RPGR*; *rd10*, retinal degeneration 10 mouse with missense point mutation in *Pde6b*.