Chapter 3

Restoring Vision to the Blind: Gene Therapy for Vision Loss: The Road Ahead

The Lasker/IRRF Initiative for Innovation in Vision Science

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Introduction

Gene therapy currently is one of the most promising treatments for genetic blindness. With the success of the phase I/II trials for Leber's congenital amaurosis (LCA) RPE65 and choroideremia (CHM), gene transfer to the retina has been shown to be technically safe, and with the potential to lead to efficacy in the treatment of retinal forms of blindness through gene augmentation in recessive or X-linked disease (MacLaren et al., 2014; Simonelli et al., 2010). A multitude of single gene disorders including retinitis pigmentosa (RP), caused by defects in over 60 different genes, remain. The challenges to bring this technology to patients in the wide spectrum of blinding disorders were discussed. There are several technical and logistical issues that are seen as hurdles in the development path that are either specific to particular approaches/indications or shared among many.

Method of Administration

There are two main routes of administration for retinal gene therapy; that is, through subretinal or intravitreal injection. Subretinal injection delivers the therapeutic vector adjacent to the retinal pigment epithelium (RPE) and photoreceptor cells by generating a retinal elevation (also referred to as bleb). By appropriately selecting a gene transfer vector, high level targeting of both cell types can be achieved. In animal models and humans, a saline formulated vector solution is absorbed, and the retina reattaches within a couple of days with limited to no consequences (Martin, Klein, & Quigley, 2002). Due to the efficiency of gene transfer, the relevance of RPE and photoreceptor gene defects in many retinal blinding disorders, and the proven safety of this injection method, it is used most commonly for outer retinal targeting. The area that is transduced is limited to the bleb; that is, the area directly around the injection site, leaving the majority of the retina untreated. Subretinal injections are more challenging technically than intravitreal injection and require surgery. Subretinal injections are not routine clinical procedures and require a highly specialized skill.

Intravitreal injections are used routinely to administer pharmacological treatment to the eye and retina. Intravitreal injections theoretically permit the vector to be exposed to a wide area of the retina from the vitreoretinal surface. However, observations in small and large animal models have shown gene transfer efficiency to be low, and primarily targeting a fraction of retinal ganglion cells suggesting a barrier for vector diffusion and/or transduction. Data in rodents indicate the inner limiting membrane (ILM) to be one barrier that traps and prevents penetration of the vector into the retina (Dalkara et al., 2009). While molecular engineering of the vector has been shown to improve this, additional methods to minimize potential hurdles pharmacologically or surgically were discussed. Additionally, once past the ILM,

diffusion through the ganglion cell layer, inner nuclear layer, and associated synaptic layers is needed to reach the photoreceptors and RPE. Novel mutations in adeno-associated virus (AAV) capsids are capable of achieving limited photoreceptor transduction following an intravitreal injection, but whether the efficacy is high enough for a therapeutic effect has not been determined (Kay et al., 2013). An intravitreal injection targets primarily retinal ganglion cells (Igarashi et al., 2013), a relevant target for optogenetic therapy for vision restoration (Bi et al., 2006) and gene augmentation in hereditary optic neuropathies (Koilkonda et al., 2014). Intravitreal injections also can be used to target Müller glial cells, which can be used to express neurotrophic agents. Besides the lower efficiency for transduction of photoreceptors and RPE, intravitreal injections are also more likely to induce a self-limiting immune response than a subretinal injection (MacLachlan et al., 2011).

Intravenous injection also has been considered for retinal gene transfer due to its less invasive nature and theoretical ability to target the entire retina through the vasculature (Bemelmans et al., 2013). However, a systemic administration of the virus would remove the advantage of treating the relatively immune privileged eye and limiting biodistribution. It also would necessitate a much higher amount of virus and, therefore, be even more likely to induce an immune response. The amount of virus needed also could pose a financial barrier, since the virus is expensive to produce. Intravenous (IV) injection could be an attractive option in mice, since they are much smaller and can be treated very young, but currently it is impractical for humans. Additionally, administering the vector systemically could lead to more off target effects when the transgene is expressed in tissues outside of the eye.

Preclinical Models to Evaluate Safety and Efficacy of Retinal Gene Therapy

Many different models are available for testing retinal gene therapy, but none of them is ideal. The mouse is the most common model used for preliminary research. Mouse strains are available with many of the mutations that affect people (Baehr & Frederick, 2009; Chang et al., 2002). If a model is not already available, a new genetically modified animal model can be developed in approximately five months. However, mice do not always recapitulate human disease phenotype even when the exact mutation is replicated in mice. For example, in humans, a mutation in *ABCA4* that results in partial inactivation of the gene leads to photoreceptor cell death (Kjellstrom, 2014). Complete loss of function results in a more severe phenotype. It would be expected that an *ABCA4* knockout mouse would result in severe retinal degeneration, but instead the mice have little or no photoreceptor cell death (Conley et al., 2012; Mata et al., 2001) and retain normal structure and function even at advanced age. Loss of *ABCA4* does result in accumulation of a waste product (A2E) in photoreceptors. In contrast, in humans, loss of *ABCA4* results in very early photoreceptor degeneration. The fact that the same mutation can cause a severe phenotype in humans, but a mild phenotype in mice illustrates the problems with using mice as a disease model. An important limitation of the mouse as model for human retinal disease is related to species-specific differences in the proportion and distribution of photoreceptor types. As noted in Chapter 2, mice do not have a fovea and relatively few cone photoreceptor cells centrally compared to humans; both are critical features in high acuity human vision. The small size of the mouse eye is another problem when evaluating gene therapy efficacy. A subretinal injection results in a proportionally larger area of the mouse retina coming into contact with the vector. An intravitreal injection has less volume to become diluted in mice than it does in humans. Also, the inner limiting membrane is thinner in mice and likely to be more permissive to the virus.

There now are transgenic models of blindness in pigs (Ross et al., 2012; Scott et al., 2014). These provide a better model of the human retina than mice. The pig eye is closer in size to the human eye, and pigs have a pseudomacula (Beauchemin, 1974). However, because of the absence of a genuine macula, the pig is not an ideal model of human retinal degeneration. Again as noted in Chapter 2, pigs are expensive to house. Additionally, degeneration time can be lengthy (Fernandez de Castro et al., 2014). Rabbits have an eye approximately two-thirds the size of a human eye, and there is a transgenic model of RP in rabbits (Kondo et al., 2009). Rabbits have long been used in ophthalmological research, but they do not even have a pseudomacula, again making the correlation with human disease less than ideal. Also, because much of the rabbit retina is avascular, subretinal injections can cause retinal damage.

Canine models of blindness also are available (Kijas et al., 2002; Miyadera, Acland, & Aguirre, 2012; Ropstad et al., 2008; Suber et al., 1993). We currently do not have any transgenic canine models of blindness, but there are several colonies of naturally occurring models of blindness in dogs. Dogs are a good model for human disease, since they have relatively large eyes, approximately two-thirds the size of human eyes, and are structurally similar to human eyes. However, they do have several significant differences from human eyes. Most importantly, they do not have a fovea. Dogs do have a cone-enriched region that can be affected by forms of macular degeneration (Beltran, et al., 2014). While not

ideal, dogs provide significant advantages over mice as a model for development of gene therapy techniques; indeed, the proof of principle studies leading to *RPE65* gene therapy were done in dogs, and gene therapy for achromatopsia has been accomplished recently in dogs (Acland et al., 2001; Beltran et al., 2012; Komaromy et al., 2010; Komaromy et al., 2013). Some models also are available in cats, such as the Abyssinian cat with a spontaneous mutation in the *CEP290* LCA gene, but with their individualism, and strong adaptive behaviors, they are more difficult to perform visual function tests on, and have not been used widely.

A compelling alternative is to verify gene transfer and vector targeting in retinas from human cadaver eyes. Methods have been established to culture human retina for up to two weeks, although it does result in loss of photoreceptor outer segments, probably due to the removal of the RPE (Johnson & Martin, 2008). Cultured retina would not model the delivery methods, since the retina is essentially bathed in the virus, but it does provide a valuable tool to validate the expression profile of the promoter and the viral tropism to the desired cell type (Fradot et al., 2011). Furthermore, gene expression and protein processing can be evaluated in the human tissue, giving a better prediction of potential problems that might arise in clinical trials, such as protein accumulation in the endoplasmic reticulum (ER) or cellular toxicity. While diseased donor eyes are difficult to access, studies on eyes from donors without apparent ophthalmic disease are thought to be highly informative. Limitations of this model include the inability to model surgical route of delivery, host immunity, and the relatively short time that retinal tissue can be cultured in relation to the onset of expression of our gene transfer vectors.

Primates are by far the best model available for the healthy human retina. Their eyes are very similar to human eyes in size and structure. Primates have a fovea as well as an inner limiting membrane with properties similar to humans (Yin et al., 2011). However, because there are no primate genetic models of blindness, they have limited use in testing the efficacy of gene therapy treatments for most retinal degenerative disease. Assessments of safety can be done in primates, but these studies make the important and possibly erroneous assumption that healthy and diseased retinal cells will respond in similar ways to the treatment. Moreover, the healthy eye is relatively immune privileged, but degeneration can affect the blood–retina barrier and allow a more robust immune response (Vinores et al., 1995).

Transgenic models for some diseases (such as Huntington's) have been produced in monkeys, but these do not include any form of retinal degeneration (Niu et al., 2014). Developing a transgenic primate model of blindness would be very expensive to create and to maintain. Most mutations resulting in blindness are rare; therefore, a transgenic monkey model would be applicable to a limited patient population with a specific subtype of blindness. There currently are 212 genes and 252 separate loci involved in retinal degenerative disease (Daiger, Sullivan, & Bowne, 2014). Even when looking at a single form of retinal degeneration, there are multiple genetic causes. Retinitis pigmentosa affects approximately 1 in 3500 people, and so far no gene has been identified that causes more than 10% of these cases. This means that at best, a transgenic monkey would be able to model 1/10 of the patients with an already rare disease (Wang et al., 2005).

What Efficacy Is Needed to Preserve or Restore Vision?

An important consideration in designing a therapy for restoring vision is to identify the threshold for success. Early treatment is correlated with better clinical outcomes. Ideally, treatment would begin before degeneration, but this generally is not possible. By the time the patient is diagnosed, significant photoreceptor loss often has occurred. When patients have lost a majority of rod photoreceptors, secondary cone degeneration begins, which, in humans, ultimately leads to gradually decreasing central vision. It is obvious that early treatment is better, but it remains largely undefined what constitutes the optimal window of treatment, and this likely varies by disease or disease gene mutation. Identifying the therapeutic window is critical in designing an efficacious treatment. Therefore, strategies that broaden the timing to achieve treatment effect are highly desirable.

According to psychophysical measurements, patients can lose 90% of their cone photoreceptors in the fovea and still have useful visual acuity (Geller & Sieving, 1993). Given the large amount of photoreceptor cell death that can occur before significant visual impairment, early genetic diagnosis is one key method to achieve this. It is possible that correcting the genetic mutation and restoring function may not be enough to halt the degenerative processes in the retina, especially if retinal rewiring has already begun. The time course of disease progression will reach a critical state in which the retina has suffered enough damage that further degeneration is independent of the initial insult. For example, a mutation in rhodopsin, which is expressed in rods, will first cause degeneration of rods. Secondary cone degeneration will follow the primary rod degeneration. Correcting the rhodopsin mutation after most of the rods have already died would not halt the

degenerative process because the cone degeneration is not the direct result of the rhodopsin mutation, but rather results from the loss of the rod photoreceptors.

A more complex question is identifying the window of opportunity for correcting RPE mutations. Gene therapy studies already have demonstrated that correcting *RPE65* will restore visual function. However, eventually in the degeneration process fundamental changes to the connectivity of the retina and activation of the glial cells may preclude correction by gene therapy. There is no doubt that gene therapy is valuable and at the very least extends the amount of time that the patient has functional vision. However the question does remain: will retinal degeneration stop after treatment or proceed with the modification of retaining photoreceptor function longer than would otherwise occur? Photoreceptor degeneration was shown to progress in the canine model and in humans, despite the sustained improvement in vision, after *RPE65* gene augmentation therapy when the therapy was administered after significant degeneration had already occurred (Cideciyan et al., 2013). These findings emphasize the need for halting the retinal degeneration process, in addition to improving retinal function. Combination therapies for retinal dystrophies should be evaluated that include correcting the underlying mutation as well as supplying trophic support to improve cell survival. The chimera created by Fulton Wong (Huang et al., 1993) is important to this. Although the chimera had patchy distribution of cells from an otherwise normal albino mouse and pigmented rhodopsin mouse, the retinal distribution of disease was as in the pigmented mouse but slower. It was concluded that although the dystrophy was due to the presence of cells with a mutation, cells with the mutation were no more or less likely to degenerate than cells without a mutation. Thus, transfecting a small proportion of cells or incomplete reversal of the genetic defect would predictably fail to reverse the degeneration completely. In some families with AD RP, visual loss occurs in the presence of many viable rods and cones, presumably due to cell dysfunction rather than cell death (Massoff, Johnson, and Finkelstein, 1981; Lyness et al., 1985; Kemp, Faulkner, and Jacobson, 1988).

Retinal Remodeling

Any gene therapy intervention or treatment will have to take into account progressive negative plasticity in the retina as photoreceptors are lost, and subsequent alterations in Müller glia and neuronal circuitry ensue (Jones & Marc, 2005). Once photoreceptors begin to die, a programmed series of alterations to metabolism and connectivity are initiated. Any interventions with gene therapies will have to take these alterations into account. Thus, exploring the previously mentioned “windows of opportunity” will be important. There may be points at which retinal rescue in the face of programmed retinal remodeling is impossible. Additionally, administration of the gene therapy via subretinal injections is complicated by retinal remodeling. During retinal degeneration, Müller glia are activated and their distal processes form a scar that isolates the retina from the subretinal space (Jones & Marc, 2005). Any treatment that involves subretinal injection will have to occur prior to Müller seal formation, since surgical detachment of the glial seal will be traumatic and likely do more harm than good. Therefore, in late stage retinal degenerations, intravitreal injections might be preferred.

Why Develop Gene Therapy for Ultra-Rare Diseases?

There is some debate about whether the value of studying rare and ultra-rare diseases balances the large cost of developing a gene therapy that will likely never be profitable. According to the United States Food and Drug Administration (FDA), a rare disease is one that affects fewer than 200,000 Americans. The European Union defines a rare disease as a disease that affects fewer than 5 in 10,000 people (Hennekam, 2011). An ultra-rare disease generally affects fewer than 1 in 50,000 people (Hughes, Tunnage, & Yeo, 2005). Among diseases causing blindness, RP affects 1 in 4000 people and LCA affects approximately 1 in 50,000. There are over 60 distinct genetic causes for RP and at least 15 different genes that cause LCA. Creating a gene therapy for any one of these genes would qualify as treating an ultra-rare disease. Developing a gene therapy for even the most prevalent form of RP would have a very limited patient population available for clinical trials. It will take a lot of effort to find the patients who are eligible for the treatment and would likely lead to logistical problems with follow-up when many patients do not live near a treatment center. Finally, given the time and cost of running a clinical trial for a slow progressing orphan retinal disease, there is a lack of economic incentive for pharmaceutical and biotech companies to develop a treatment with a limited number of patients who would purchase the final product, especially if the trial itself depletes a significant number of the treatable population before it reaches market and distribution. The FDA Office of Orphan Products Development (OOPD) provides incentives for sponsors to develop products for rare diseases and has been used to bring more than 45 products to marketing approval

since 1983 (FDA, 2014). The EU also offers a range of incentives to encourage the development of medicines for rare diseases, including tax credits on clinical research, reduced charges, and ten years of market exclusivity (EMA, 2014).

However, gene therapy provides what no other therapy can: a permanent solution to a genetic problem that requires only one administration. The eye is an ideal place to develop gene therapy. The relative immune privilege in the retina removes some of the negative effects possible from treatment with a viral capsid. The relatively small area of the retina is also attractive since it can be treated with a single low volume (<1 mL) injection into each eye as opposed to a muscular or liver disease that requires a higher volume and multiple injections. The blood–retina barrier ensures that the majority of the vector remains in the eye, although a small amount likely escapes. The spatial isolation of the eye prevents the protein from being expressed in other tissues even if a ubiquitous promoter is used.

Treating rare and ultra-rare diseases in the retina may provide the building blocks for other gene therapy trials. Validating the safety of the capsid used in the retina allows other trials that use the same capsid to utilize safety data gained in the first trial. Validating a capsid/promoter combination to target certain cell types (photoreceptor or RPE) could lead to a “cut and paste” approach, in which a different gene could be inserted into the vector with the reasonable expectation that it would be expressed in the desired cell type. This type of approach might reduce the extensiveness of some of the IND-enabling studies and even phase 1 clinical studies, leading to time and cost savings that could make developing a gene therapy for other related diseases easier and cheaper than the first therapy, effectively lowering the barriers.

New Approaches and Future Studies

1. The gene addition or augmentation studies in LCA due to the *RPE65* defect has spurred the development and exploration of new technologies and therapeutic approaches that aim at addressing limitations of the first generation of retinal gene therapies. Indeed, a significant effort has been devoted to the discovery, characterization, and optimization of vector technologies that alter tropism or increase gene transfer efficiency by altering, for example, the AAV capsid proteins or modifying the genomic structure of the viral vector genomes (Dalkara et al., 2009; Kay et al., 2013; Natkunarajah et al., 2008; Vandenbergh & Auricchio, 2012). To address the limited therapeutic window of gene augmentation strategies, two approaches have generated substantial interest and are discussed in separate chapters: optogenetic therapy for vision restoration and neuroprotective strategies to delay retinal degeneration. These also have been reviewed extensively by Sahel and Roska (2013).
2. A more recent prospect is that of corrective genome editing therapy; that is, editing the patient’s own genome to eliminate disease-causing mutations, as an attractive theoretical modality of gene therapy. In current therapies, the gene either remains episomal and, therefore, is not integrated into the patient’s genome, or the gene is inserted randomly into the genome. By targeting the mutated gene in situ, at the wild type locus, we theoretically could correct the mutation while leaving the gene under the control of the endogenous promoters and enhancers; genes that exceed the packing capacity of viral vectors also could be targeted.
3. Several methods currently are being developed for genome editing, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the newly developed clustered, regularly interspaced, short palindromic repeat RNA-guided nucleases like the CRISPR/Cas9 system (Gaj, Gersbach, & Barbas, 2013). Each relies on the delivery of a gene-editing system into the affected cells using viral mediated gene therapy. All three systems function by inducing double-stranded DNA breaks at specifically targeted location in the genome. The DNA break can be repaired in a way that prevents expression of the target gene by inducing a missense or nonsense mutation (Ran et al., 2013; Sung et al., 2014). This strategy would completely correct the mutated gene, but is more challenging, since it involves the additional step of incorporating the template sequence. The site then can be repaired by the error-prone nonhomologous end-joining, with the intended result of a missense or nonsense mutation that will prevent expression of the targeted gene (Ran et al., 2013; Sung et al., 2014). Alternatively, homologous repair can be used to incorporate a template sequence to correct a genetic mutation (Ran et al., 2013; Rouet, Smih, & Jasin, 1994).

The ZFNs, TALENs, and the CRISPR/Cas9 systems differ in the strategy they use to target the desired DNA sequence and the method used to cleave the DNA. ZFNs were the first form of directed genome editing used as a gene therapy (Urnov et al., 2005). ZFNs target specific genetic sequences using combinations of zinc fingers, which are approximately 30 amino acids in length and target three base pairs each. By using an array of 3 to 6 zinc-fingers, the ZFN can target a sequence of 9 to 18 base pairs long and usually are used in pairs (Mani et al., 2005; Miller et al., 2007). The DNA break is induced by the FokI nuclease (Ramalingam et al., 2011). ZFNs are large, difficult to design and can be difficult to target to the desired site (Kim & Kim, 2014). TALENs also use the FokI nuclease, but they use

a different method to target the desired DNA sequence (Miller et al., 2011). TALENs are made up of 33 to 35 amino acid modules that target a single nucleotide (Deng et al., 2012). These modules can be combined to target specifically the desired location in the chromosome (Zhang et al., 2011). TALENs are much larger than ZFNs, making them even more difficult to deliver to the target cells (Gaj et al., 2013). Both ZFNs and TALENs depend on a coding that relates their amino acid binding sequence to a specific nucleotide sequence. The simpler code of the TALEN makes them cheaper to develop and provide a more flexible platform that can be modified to target more sites than ZFNs are capable of targeting. CRISPR is the newest form of genome editing (Yin et al., 2014). As opposed to ZFNs and TALENs, the CRISPR/Cas9 system targets the desired DNA sequence using a guide RNA that is approximately 20 nucleotides long, making it by far the smallest and easiest to administer platform. It uses the Cas9 nuclease, which, unlike FokI, does not require dimerization to function (Jinek et al., 2012). Furthermore, the guide RNA is relatively easy to design and inexpensive to produce (Sander & Joung, 2014). The potential for off-target binding of the guide RNA still is in question and strategies are being developed to increase the specificity (Fu et al., 2014; Kucsu et al., 2014).

4. There are some key hurdles to overcome before CRISPR can be used therapeutically to fix mutated genes. Perhaps most importantly, the corrected gene segment and *Cas9* would have to be transduced into a large number of cells to be effective. Also, *Cas9* could cause some off target cleavage, especially at high concentrations and when present in the cell for an extended duration. One possible solution would be to design a self-targeting *Cas9* that will cleave itself at a lower rate than the target gene or have inducible expression of the CRISPR/Cas9 components and the nuclease in particular. This would allow the enzyme to be active for a very limited time and limit the off target effects. Another potential problem for retinal gene modification is that CRISPR requires endogenous DNA repair mechanisms to perform genome editing (Auer et al., 2014). DNA repair generally takes place during DNA replication. Since postmitotic neurons do not undergo DNA replication, traditional repair mechanisms do not occur. Initially, it was believed that postmitotic neurons did not undergo DNA repair; however, it now is known that active loci still undergo repair (Lee & McKinnon, 2007). Since the genes that would be targets for genome editing are actively transcribed in the target cells, CRISPR should be capable of genome editing at the target loci.

CRISPR is especially promising for targeting gain-of-function mutations in which silencing of the mutated allele would be sufficient to preserve the cell. Using *Cas9*, the sequence of the gene could be disrupted in a way that would prevent translation of that allele. The challenging aspect of the therapy would be to make the targeting sufficiently specific to target only the mutated allele.

5. Alternatives to traditional gene therapy, where the mutated gene is corrected, are more generic gene therapy approaches that would be broadly applicable regardless of the actual gene that is mutated. One such approach is optogenetics, which is covered in Chapter 2. Optogenetics is a gene therapy approach in which a light-sensitive protein is inserted into either remnant photoreceptors or inner retinal cells to restore some level of light perception after photoreceptor degeneration. This approach would result in a lower level of visual function than traditional gene therapy approaches that seek to prevent photoreceptor degeneration, but the patients it could benefit would not be limited to those suffering from specific and very rare diseases. Furthermore, these therapies would have a larger window of opportunity, since optogenetics could be effective even after complete photoreceptor cell death.
6. Another idea is to knock down the neural retinal leucine zipper (Nrl) protein somatically in patients who have a mutation that primarily affects rods. When Nrl is absent, rods develop a cone-like morphology. This approach was tested in rd1 mice that normally lose all rod photoreceptors by one month of age. When Nrl was knocked out, photoreceptor cell death was prevented (Montana et al., 2013). The rods did not function properly, but they did not degenerate. Preventing cell death, even without preserving rod function would be extremely beneficial to patients with rod-cone dystrophies, since it would prevent or delay the secondary cone degeneration, thereby preserving normal daylight vision. However, mutations in Nrl are associated with retinal degeneration, although on a slower time course (Yoshida et al., 2004). Therefore, knocking out Nrl as a treatment for blindness would most likely result in a slower degeneration, but not a halt to degeneration.
7. One problem with the way research currently is done is that knowledge is not readily shared and findings are insufficiently cross-validated by multiple groups, particularly on the available gene transfer tools. A platform to share information and experience other than through journal articles would be welcomed and may limit redundant effort, especially in reporting negative results (e.g., *AAVX* does not transduce horizontal cells following subretinal injection at a certain dose in a certain model). However, there is no easy way to share technical issues and solutions in a way that is

accessible and convenient to search, and maintenance of such a platform would come at cost and significant effort. One model or option for achieving this is Addgene (www.addgene.com), a nonprofit organization that makes plasmid reagents accessible and provides information on those.

Conclusion

Gene therapy is highly promising as a solution for genetic diseases without therapeutic alternatives. Using this paradigm in vision loss is even more attractive due to the accessibility and relative safety of the eye. A variety of new approaches build on the gene therapy platform, including gene replacement for monogenetic diseases, genome editing to knockout dominant mutations and generic approaches, like optogenetics. Genome editing in particular is opening up new opportunities in disease modeling and treating gain-of-function mutations. The main hurdle facing gene therapy is the high cost associated with developing treatments and bringing them to clinical trials in the context of orphan diseases, and the barriers presented by the lack of knowledge sharing. Building a better platform for data sharing would go a long way toward enabling the development of new gene therapies.

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