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Precision genome editing in the eye

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CRISPR-Cas-based genome editing technologies could, in principle, be used to treat a wide variety of inherited diseases, including genetic disorders of vision. Programmable CRISPR-Cas nucleases are effective tools for gene disruption, but they are poorly suited for precisely correcting pathogenic mutations in most therapeutic settings. Recently developed precision genome editing agents, including base editors and prime editors, have enabled precise gene correction and disease rescue in multiple preclinical models of genetic disorders. Additionally, new delivery technologies that transiently deliver precision genome editing agents in vivo offer minimized off-target editing and improved safety profiles. These improvements to precision genome editing and delivery technologies are expected to revolutionize the treatment of genetic disorders of vision and other diseases. In this Perspective, we describe current preclinical and clinical genome editing approaches for treating inherited retinal degenerative diseases, and we discuss important considerations that should be addressed as these approaches are translated into clinical practice.

retina | eye | genome editing | retinal degeneration

Inherited retinal diseases (IRDs) are a genetically heterogeneous group of blinding disorders characterized by a progressive degeneration of the photoreceptors as well as the retinal pigment epithelium (RPE) (1). These disorders affect ~1 in 3,000 individuals worldwide and profoundly impact patients' quality of life (2). IRDs are caused by mutations in genes that are critical for development and/or function of the retina or RPE, and more than 270 causative genes have been identified (2). IRDs display a broad spectrum of disease subtypes with variable onset, severity, rate of progression, topography of the retinal involvement, and mode of inheritance, making these diseases more challenging to treat than many other diseases (2, 3).

Over the past two decades, major advances in gene therapy have engendered new hopes for successful treatment of these IRDs. The eye is a particularly attractive target for gene therapy, due to its easy accessibility, immune-privileged status, and compartmentalized structure and the advantage of using the contralateral eye as a control (4). In 2017, the US Food and Drug Administration (FDA) approved the first gene augmentation therapy for treatment of an IRD (5). This therapy, sold under the brand name Luxturna (voretigene neparvovec-rzyl), is intended to treat patients with biallelic loss-of-function mutations in *RPE65*, a gene responsible for encoding a critical enzyme in the visual cycle (RPE-specific 65-kDa protein) (6). Luxturna involves injecting an adeno-associated virus (AAV) that delivers a copy of the normal *RPE65* complementary DNA (cDNA) to the patient's RPE cells, thereby compensating for the missing enzyme (Fig. 1*A*). The AAV is administered as a one-time treatment to each eye, no fewer than 6 d apart (7). The approval of Luxturna brought hope to patients suffering from inherited retinal degeneration and further opened the door to the potential of other gene therapies.

While there is consensus that *RPE65* gene augmentation therapy improves the visual acuity of patients, the durability of therapeutic benefit is unknown. The clinical studies published in 2013 and 2015 reported subsequent decline in visual sensitivity and continuous progression of photoreceptor degeneration after 3 y following treatment (8–10). These studies reported that the natural rate of photoreceptor degeneration due to *RPE65* mutations was not modified by the gene therapy when treatments were initiated after the onset of degeneration, and, therefore, the restored visual sensitivities started waning in the long term (8–10). However, clinical studies published in 2019 and 2021 show that visual function improvements after gene therapy were sustained up to 4 y to 7.5 y, with observation ongoing (11–13). Given the short history of the *RPE65* gene augmentation therapy, it is unclear how long the treatment effects will last and why some patients have relapsed over a few years.

The reason for the declining treatment effect is not known, but several possible contributing factors include 1) unmet physiological demand from exogenously expressed RPE65 (8–10), 2) silencing of the exogenous transgene over time, and 3) cellular stress from the products of the mutant allele (14, 15). These limitations to existing gene augmentation

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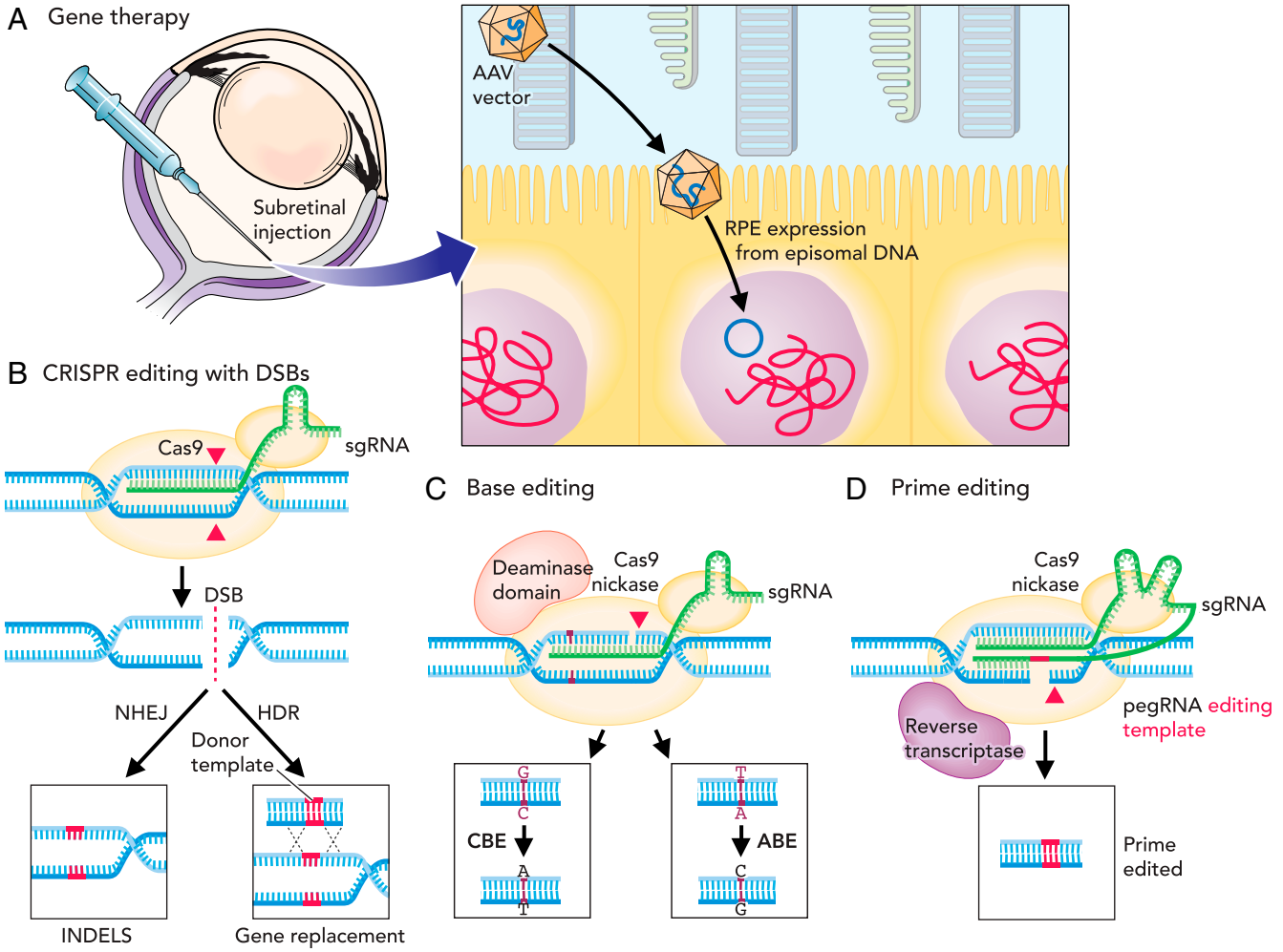

Fig. 1. Conventional gene augmentation therapy and CRISPR-based genome editing approaches. (*A*) Gene augmentation therapy involves an AAV that carries and delivers a copy of the normal *RPE65* cDNA to the RPE. (*B*) CRISPR-Cas-nuclease-mediated genome editing generates DSBs that are repaired by either NHEJ or HDR. NHEJ is the primary pathway for DSB repair throughout the cell cycle. Uncontrolled nucleotide insertions or deletions often occur as a result of NHEJ, whereas desired nucleotide changes specific to a donor DNA repair template are achieved via HDR. (*C*) BEs install targeted single-nucleotide conversions using either cytidine deaminase (CBE) or adenosine deaminase (ABE) domains tethered to a Cas9 nickase with an sgRNA. CBEs perform targeted C•G-to-T•A conversions, and ABEs perform targeted A•T-to-G•C conversions. (*D*) PEs utilize a reverse transcriptase tethered to a Cas9 nickase to write new DNA sequences into the target locus. Use of the Cas9 nickase avoids the formation of a DSB. A pegRNA, an extended sgRNA that contains the template sequence for reverse transcription, is utilized for nucleotide synthesis at the target locus. Red triangle indicates the site of DNA strand break.

approaches have motivated the development of additional strategies for treating IRDs.

Genome editing technologies have shown great promise for treating the root causes of genetic disorders. Clinical trials are already underway that use CRISPR-Cas-based technologies for treating cancers, blood disorders, chronic infections, protein-folding disorders, and eye diseases (16). Therapeutic applications of nucleases including zinc-finger nucleases, TALE nucleases, and CRISPR-Cas9 have a primary focus on target gene disruption, as uncontrolled mixtures of insertions and deletions (indels) are the most common outcomes of the DNA double-strand breaks (DSBs) made by nucleases (17). Most recently, precision genome editing agents, including base editors (BEs) and prime editors (PEs), have enabled efficient and precise target gene correction, rather than gene disruption, in various therapeutic settings, including mouse models of IRDs (18–21). Precise target gene correction greatly expands the potential therapeutic applications of genome editing technologies, since most genetic disorders cannot be treated by gene disruption (22–25). Here, we discuss progress toward using genome editing for treating IRDs and important considerations for robust clinical translation.

CRISPR-Cas Nuclease Editing in Animal Models of IRDs

CRISPR-Cas nucleases are powerful tools for genome engineering that generate targeted DSBs in genomic DNA (22, 25). These nucleases can be reprogrammed to target different genomic loci by changing the sequence of a portion of the single-guide RNA (sgRNA) molecule loaded within the enzyme (22, 25). DSBs are mended by one of two major repair pathways: end joining and homology-directed repair (HDR) (26). End-joining pathways, which are further divided into nonhomologous end joining (NHEJ) and alternative end joining, re-ligate the broken ends of DNA in a template-independent manner, often resulting in a heterogenous pool of insertions and deletions (26). In contrast, HDR pathways use a donor DNA template to repair the break, leading to targeted gene integration (26).

Most early studies demonstrated correction of IRD-causing mutations in cells or animal models by using HDR (Fig. 1*B*). In this process, a DSB is generated at the mutant genomic locus, and a donor DNA molecule (containing the desired, corrected sequence flanked by regions of homology to the target site) is provided to serve as a template as the cell repairs the DSB (22, 25). Using HDR approaches, researchers demonstrated the correction of mutations in the *RPGR, PDE6β, MAK, N2RE3, RPE65,* and *RHO* genes in patient-derived induced pluripotent stem cells (iPSCs) or mouse models (27–30). However, the major drawback of HDR-based editing strategies is that desired HDR products are typically far outnumbered by unwanted editing byproducts (31). HDR, which is restricted to S/G2 phases of the cell cycle, is far less efficient in nondividing cells such as photoreceptors and RPE cells compared to the end-joining pathway, which is active in both dividing and nondividing cells (32–34). Therefore, the undesired byproducts that arise from end-joining pathways usually outcompete precise corrections (Fig. 1*B*). For these reasons, nuclease-mediated genome editing is poorly suited for precisely correcting pathogenic mutations in most therapeutic settings.

Nuclease-mediated editing is, nonetheless, useful for applications in which uncontrolled mixtures of indels are tolerated or desired. This includes strategies in which disrupting gain-of-function or dominant negative alleles with indels can lead to therapeutic benefits. Researchers demonstrated applying this approach in animal models of autosomal dominant retinitis pigmentosa (adRP) by selectively ablating a rhodopsin (*Rho*) gene carrying a dominant S334ter mutation in rats or a P23H mutation in mice (35, 36). This approach, however, is conditionally feasible on the premise that Cas9 and the designed sgRNA can selectively disrupt the mutant allele but not the wild-type (WT) allele. Moreover, the development of many different sgRNAs to target each mutation specifically presents a fiscal challenge in drug development.

To address these problems, Tsai et al. (37) developed a two-pronged ablate-and-replace strategy for the treatment of adRP resulting from mutations in *Rho*. The ablate-and-replace strategy first destroys the expression of the endogenous mutant and normal *Rho* gene in a mutation-independent manner via a CRISPR-based gene deletion, and then restores the expression of WT protein via exogenous cDNA delivery (37). Using this approach, Tsai et al. demonstrated amelioration of disease progression through improved retinal structure and function in two adRP mouse models (37).

To improve the efficiency of precise genomic repair in nondividing cells, Suzuki et al. (38) developed a homology-independent targeted integration, which relies on NHEJ for targeted transgene integration. In this method, a donor template lacks homology arms, but contains Cas9 cleavage sites at both ends. When Cas9 cleaves at both the donor and the genomic target sequence, this allows the donor sequence to be integrated into the genomic DSB site via NHEJ (38). Using this approach, the group demonstrated restoring a visual function in a rat model of retinitis pigmentosa by inserting a missing exon from the *Mertk* gene (38).

Nuclease-mediated editing was also used to correct a splicing defect in the *CEP290* gene, which is caused by the most common mutation associated with Leber congenital amaurosis (LCA) type 10 (LCA10) (39). Mutations in *CEP290* are one of the most common causes of LCA10, accounting for up to 20% of all LCA cases (40). Although the function of CEP290 is not clearly understood, it is thought to play a structural role in the cilia of photoreceptor cells (41). The mutation, IVS26 c.2991 + 1655 A > G in intron 26 of the *CEP290* gene, generates a novel splice donor, resulting in aberrant protein splicing and the inclusion of an additional 128-bp cryptic exon in the coding sequence of the transcript (39). Researchers employed *Staphylococcus aureus* (*S. aureus*) Cas9 and two sgRNAs to induce DSBs at both ends of the region of DNA to excise the intronic mutation and restore normal splicing between exons 26 and 27 in mice (39). Delivery of these components into mice using a single-AAV approach led to increased expression of WT CEP290 and concomitant decrease in expression of the defective CEP290 (39). Furthermore, experiments in human retinal explants did not detect any off-target editing at over 100 candidate sites (39). This successful preclinical study led to clinical trials for treating LCA10 patients, as described below.

First CRISPR-Cas Nuclease Clinical Trial for the Treatment of LCA10

In March 2020, Editas Medicine announced that the first patient was dosed with an in vivo CRISPR-Cas nuclease therapy to treat an IRD. This therapy, EDIT-101, uses an AAV-delivered Cas9 and two sgRNAs to remove an intronic point mutation in the *CEP290* gene (the so-called IVS26 mutation), which leads to incorrect splicing of the *CEP290* transcript and causes LCA10 (39). Similar to the preclinical study, EDIT-101 employs a single AAV containing two human U6 polymerase III promoter-driven sgRNAs and human G protein–coupled receptor kinase 1 promoter-driven *S. aureus* Cas9, which is smaller than *Streptococcus pyogenes* (*S. pyogenes*) Cas9 (39).

The promising results from preclinical assessment of EDIT-101 supported a subsequent phase 1/2 clinical trial, BRILLIANCE, which included 11 adult patients (NCT#03872479). Although the posttreatment assessment of the patients is still ongoing, preliminary clinical results, presented at the 2021 European Society of Gene and Cell Therapy Annual Congress, reported a favorable safety profile and encouraging early signs of efficacy in the patients. A comprehensive update on safety and efficacy of the treatment is anticipated by the second half of 2022, after 12 mo of follow-up evaluations. Recently, the company accomplished a significant milestone by expanding the administration of EDIT-101 to pediatric patients (NCT#03872479), which is an important step toward bringing potentially life-changing treatments to children with IRDs.

Treating IRDs by Precision Genome Editing

While nuclease-mediated editing is promising for applications that benefit from targeted gene disruption, recently developed precision genome editing strategies are better suited for precise gene correction in therapeutically relevant cells, which often do not express the cellular machinery required to support HDR. In particular, numerous researchers have demonstrated that BEs and PEs can

precisely correct a variety of disease-causing mutations with minimal undesired editing byproducts across multiple therapeutically relevant cell types and organisms. BEs and PEs have already been used to treat mouse models of IRDs, and therefore are promising candidates for future clinical translation.

Base Editing in the Eye. Four years after CRISPR-Cas nucleases were introduced, base editing emerged in 2016 as a more precise genome editing technology that can efficiently install single-nucleotide conversions with minimal undesired indel byproducts and without generating DSBs (20). BEs are engineered proteins comprising a programmable DNA-binding protein such as a disabled Cas nuclease or a TALE repeat array tethered to either a cytidine deaminase (cytosine BE [CBE]) or a laboratory-evolved deoxyadenosine deaminase (adenine BE [ABE]) (19, 20). When a BE and sgRNA are introduced into cells, the resulting ribonucleoprotein (RNP) complex binds the target genomic DNA, displacing a single-stranded DNA bubble that can be deaminated by the tethered deaminase domain (19, 20). CBEs or ABEs deaminate C•G or A•T base pairs to yield U•G or I•T intermediates, respectively. Nicking the nondeaminated strand biases cellular DNA repair to replace the unedited strand, thereby resolving the mismatched U•G or I•T intermediates into stable T•A or G•C outcomes, respectively (Fig. 1*C*) (19, 20). Base editing has shown promising outcomes in various preclinical models of genetic disorders in muscle, skin, heart, and liver tissues (23, 42–46).

The first in vivo application of base editing to treat an IRD was demonstrated in the *rd12* mouse model of LCA type 2 (LCA2), which harbors a single nonsense mutation in exon 3 of the *Rpe65* gene (the same gene supplied in the approved Luxturna gene augmentation therapy) (47). Subretinal delivery of a lentiviral vector encoding an ABE and appropriate sgRNA into adult *rd12* mice corrected the target mutation precisely with up to 29% efficiency, and with less than 0.5% indel formation as well as undetectable off-target editing. Treated mice displayed restored expression of functional RPE65, along with restored visual cycle and retinal and visual function; they could discriminate visual changes in terms of direction, size, contrast, and spatial and temporal frequency (47). Separately, Jang et al. (48) demonstrated that ABE:sgRNA RNPs could be delivered to *rd12* mice using lipofectamine when administered subretinally, documenting nonviral base editing in the eye. They demonstrated that a transient expression of base editing components delivered via lipofectamine achieved a maximum correction efficiency up to 5.7% without detectable bystander editing. Treated mice showed a significant increase in *Rpe65* messenger RNA (mRNA) and expression of RPE65 proteins.

In a subsequent study, base editing therapy was shown to provide long-lasting retinal protection and prevent vision deterioration in mice with LCA (49). Maintaining the improved visual sensitivity has been a long-standing challenge with current *RPE65* gene augmentation therapy, as several long-term clinical studies have reported that patients showed progressive retinal degeneration and consequent decline in visual acuity a few years after treatment (8–10). Therefore, the ultimate goal of treatment should be the protection of photoreceptors to prevent further vision deterioration. In this more recent study, base editing was found to restore function and prolong survival of cones even in the advanced stage of retinal degeneration, which had been considered to be beyond the therapeutic window (49). Both cone function and population were remarkably preserved up to 6 mo following treatment, in contrast to the extensive cell death that occurred in untreated mice by 5 wk of age. Also, single-cell RNA sequencing analysis showed significant up-regulation of genes that are crucial for cone function and survival in treated mice.

Several factors may play a role in the robust rescue of cone photoreceptors by base editing, which is not achieved by *Rpe65* gene augmentation. First, base editing installs a permanent correction in the genome, thereby eliminating the concerns for declining transgene expression over time (9). Secondly, base editing to correct the endogenous locus allows more physiologically regulated gene expression, as the corrected gene is controlled by the endogenous promoter and transcription factors (50). Lastly, precise endogenous correction stops the expression of a truncated, dysfunctional protein, alleviating the potential stress on cells. Taken together, these factors likely contributed to the sustained rescue of cone photoreceptors observed in ABE-treated LCA2 mice.

Prime Editing in the Eye. Prime editing was introduced in 2019 and substantially expanded the scope of pathogenic genetic variants that are correctable in principle without requiring double-strand DNA breaks or generating excess gene-disrupting indels (18). BEs, despite their ability to correct all transitions and some transversion point mutations, cannot perform all possible single-nucleotide conversions. PEs are capable of installing any of the 12 possible base substitutions as well as small insertions, deletions, and combinations thereof (18). PEs consist of a Cas9 nickase fused to a reverse transcriptase domain; they use prime editing guide RNAs (pegRNAs) both to direct the PE to the target locus and to serve as an RNA template for reverse transcription, primed by the nicked genomic DNA. This design enables PEs to directly write a desired DNA sequence into genomic loci of interest, without creating DSBs, and without the risk of unwanted bystander editing (18) (Fig. 1*D*).

Jang et al. (51) demonstrated the first in vivo application of prime editing to treat an IRD in *rd12* mice, the model of LCA2 that was used in previous base editing studies. Although the *rd12* disease-causing mutation is a G•C-to-A•T transition mutation (correctable by ABE), Jang et al. (51) demonstrated that dual-AAV delivery of a PE and pegRNA could also correct this mutation. Prime editing corrected ~28% mutant alleles of the RPE cells that were transduced. Most importantly, there were no unintended edits, substitutions, or indels observed near the target site. As prime edits are dictated entirely by the pegRNA sequence, PEs do not induce any bystander edits, representing an advantage over certain BEs that might deaminate multiple cytosines or adenines within an activity window (18). These results indicate that prime editing, in addition to base editing and CRISPR-Cas nuclease approaches, is a promising strategy for

therapeutic genome editing for IRDs and other genetic diseases. Future applications of prime editing in animal models of other IRDs caused by transversion mutations, small-sized insertions, or deletions would further support its immense potential therapeutic utility.

Future Steps and Challenges: Safety, Efficiency, and Precision

Advances in precision genome editing technologies have opened up unprecedented opportunities for genome editing therapies. However, applying these technologies in the clinic requires overcoming several hurdles, including safety and efficiency. Evaluating the potential risks and benefits of genome editing in preclinical and clinical studies will provide insights for the use of genome editing as a therapeutic tool. In the following, we discuss recently published studies reporting new technologies and findings aimed at addressing limitations of current genome editing therapies.

Overcoming Persistent Expression of the Genome Editor. Most preclinical and clinical studies have used viral vectors, AAVs or lentiviruses, to deliver cDNAs that encode genome editing agents to target tissues. Despite the relatively favorable safety profiles of BEs and PEs, prolonged expression of these agents from the delivered cDNA increases the chance of off-target editing in the target cells (52, 53). In addition, viral delivery carries the risk of integrating viral DNA into the host genome, which could increase the likelihood of oncogenesis (54, 55). Moreover, persistent expression of the editing machinery could give rise to antiviral immune responses in the long term.

To overcome these drawbacks of viral delivery, extensive efforts have been devoted to developing alternative safe and efficient delivery approaches (56–60). Recently, Banskota et al. (61) developed engineered viral-like particles (eVLPs), which enable in vivo delivery of genome editing agents as RNPs instead of as nucleic acids. This approach eliminates the risks of viral DNA integration and persistent expression, as the delivered RNPs exhibit a very short lifetime in target cells. A single subretinal injection of BE-packaging eVLPs into LCA2-model mice achieved equivalent on-target editing efficiencies but reduced off-target editing compared to lentiviral delivery (61); eVLP treatment also resulted in a remarkable restoration of visual function. These results are encouraging, as the therapeutic potential of eVLPs can be expanded further by developing eVLPs with different tissue tropisms. The eVLPs combine key advantages of both viral delivery and nonviral delivery and are a promising new approach for transiently delivering precision genome editing agents in vivo.

Efficient Delivery to Different Cell Types in the Eye. Genes that are implicated in IRDs are expressed in many different cell types in the retina and RPE, including rods, cones, RPE cells, bipolar cells, and retinal ganglion cells (62) (Fig. 2). The majority of known genetic mutations impact the photoreceptors (*ABCA4, RHO, GUCY2D, RDH12,* and *USH2A*) and RPE cells, which are in the outer layer of the eye. To target photoreceptors or RPE cells, subretinal injection is the standard mode of delivery. However, these injections,

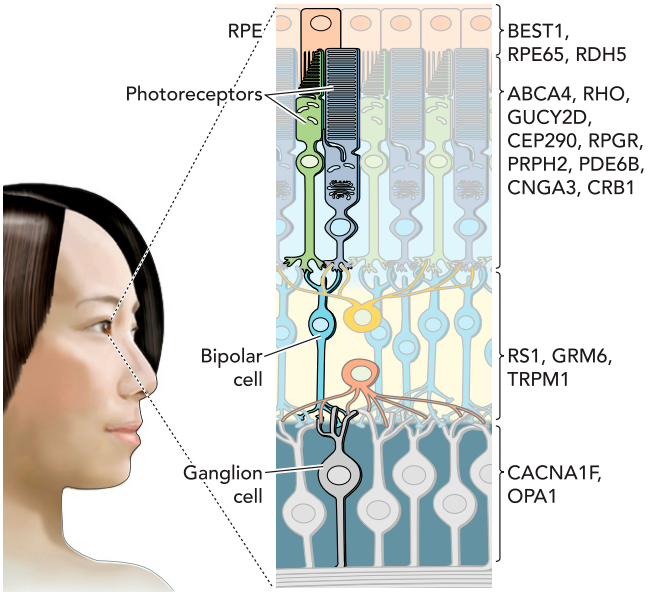

Fig. 2. Genes implicated in IRDs. Mutations in the genes predominantly expressed in the RPE, photoreceptors, bipolar cells, or retinal ganglion cells cause retinal degeneration. Depending on the type and site of mutation, selection of an appropriate genome editing agent and delivery vehicle can be optimized to target these genes efficiently.

compared to intravitreal injections, carry a higher risk of complications, such as collateral damage to the fragile retina and detachment of the retina from the RPE (63). To mitigate the latter risk, only small volumes of vector are administered, to limit detachment to only a small part of the retina (64). The current FDA recommendation for Luxturna treatment indicates subretinal injection with a total volume of 0.3 mL for each eye (65). The product is injected slowly at 2 mm away from the center of the fovea until an initial subretinal bleb is observed (65). Due to limited spreading of the vector out of the subretinal bleb, only a small portion of the affected retina can be treated in this way (64). Therefore, there is high demand for new delivery vehicles that can cross multiple biological barriers and transduce diseased cell types with therapeutic efficiency. Indeed, recent advances in AAV engineering technology have supported the development of enhanced viral capsids with interesting transduction properties. Researchers have generated numerous libraries through directed evolution of AAV capsids, a strategy to harness genetic diversification and selection processes, enabling discovery of novel synthetic capsids with desired characteristics (66). These efforts led to a unique capsid that facilitates targeting of photoreceptors in mice and nonhuman primates via the less invasive route of intravitreal administration, suggesting that this capsid could be used in future clinical trials of gene therapy (67, 68). Combining these engineered AAVs with precision genome editing agents as the delivered cargos will accelerate our ability to treat a broad range of IRDs.

Immunogenic Response. Investigating the immune response to intraocular injection of Cas9-associated machinery is crucial for clinical trials. Although the eye is considered an immune-privileged organ, intraocular inflammation remains a major cause of vision loss (69). The eye contains

antibodies, cytokines, and resident immune cells that may lead to immunological activation and inflammation (70, 71). Toral et al. (72) analyzed the presence of Cas9-reactive antibodies in serum and vitreous fluid biopsies from 13 adult human subjects and mice. There was a high prevalence of preexisting Cas9-reactive antibodies in serum but not in the eye, reflecting a lower risk of immune reaction in human eyes. However, a subset of mice developed *S. pyogenes* Cas9-reactive antibodies in the vitreous fluid after intraocular infection with *S. pyogenes*. These findings warrant further research to determine whether intraocular Cas9 exposure from genome editing increases the risk of inflammation or impedes therapy.

Besides Cas9, patients may also develop antibodies against the protein that is newly expressed from a repaired gene, as the protein could be recognized as foreign by the immune cells. Also, AAV vectors themselves have been reported to elicit immune responses, as AAVs could enter the vitreous fluid and subsequently cause inflammation in the eye (73, 74). It has been documented that AAV is recognized by the immune system and causes gene therapy–associated uveitis in a dose-dependent manner (73, 74). Further research is needed to evaluate all possible immune-related risks of genome editing and to identify potential ways to mitigate such risks.

Improving the Precision and Efficiency of Editing. Despite the development of precise genome editing tools, the application of genome editing as a therapy carries risks of making undesired mutations in the genome, which could do more harm than good to patients. Also, the efficiency of editing must achieve a certain threshold to elicit therapeutic benefits in patients. Therefore, improving the precision and efficiency of genome editing is crucial for clinical translation. Although the advent of PE enabled higher on-target editing rates up to 50% and lower off-target editing in studies (18), da Costa et al. (75) have highlighted limitations with current technology and the importance of developing more precise and efficient genome editing tools. First, there is a large variability in the editing rates across cell types, loci of target genes, and characteristics of the PEs (76–80). Moreover, the editing efficiency is shown to be notably lower when translated from in vitro to in vivo (81, 82). Another limitation includes the need for better tools and technologies to thoroughly evaluate off-target editing frequency in a genome-wide manner instead of analyzing the top predicted off-target sites (75). Lastly, the large size of the PE machinery poses challenges for efficient in vivo delivery. PEs can be delivered to target cells as a split dual-AAV approach, which leads to lower editing efficiency (82). As future research will likely overcome these limitations, precision genome editing such as prime editing holds great promise as a new effective therapeutic paradigm for treating various genetic diseases.

Future Clinical Application of Precision Genome Editing in Patients. Although precision genome editing is an attractive treatment approach in principle, its broad clinical implementation is impeded by the tremendous amount of time and cost associated with designing personalized therapies based on the unique genetic makeup of each patient. From a practical perspective, it not feasible to screen hundreds of BE and sgRNA combinations using cells derived from each patient, assess a thorough safety profile, and obtain FDA approval to develop the strategy into a broadly applicable treatment with a reasonable price tag. To address this practical barrier, there needs to be an efficient algorithm that can formulate a treatment tailored to a specific mutation by, for example, utilizing deep machine learning models to generate the most efficient editing strategy (83, 84) (Fig. 3). The in silico–designed editing strategy could be further validated in differentiated patient-derived fibroblasts or subsequently iPSC-derived organoids.

The iPSC-derived organoids, which comprehensively recapitulate the structure and functionalities of their counterpart organs, hold great promise for facilitating the development of therapeutic treatment of inherited diseases and personalized medicine (85, 86). Unlike two-dimensional cell cultures, organoids allow us to understand intercellular interactions and the overall effect of treatment on cells in the context of

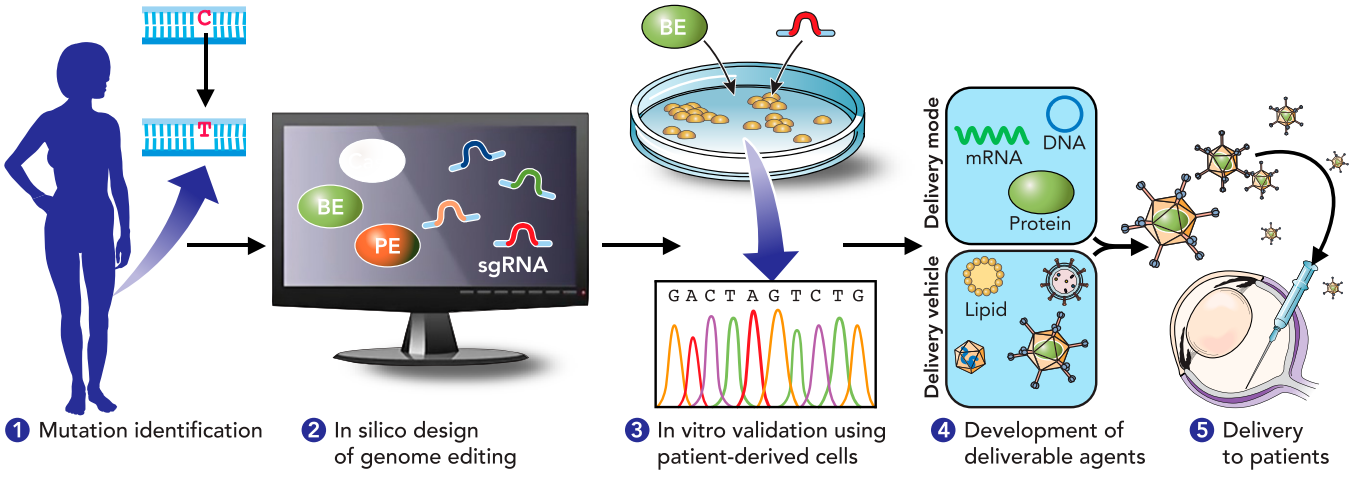

Fig. 3. Schematic representation of a genome editing approach for precision medicine. Personalized genome editing therapy would begin with the identification of a patient's pathogenic mutation, followed by a computational prescreening process to generate a candidate library for experimental validation using a patient-derived cell line. The final custom-designed editing agent could be delivered to the patient as nucleic acid, mRNA, or RNP by choosing from various vehicles including AAV, lentivirus, engineered virus-like particles, lipid nanoparticles, gold nanoparticles, or others.

their microenvironment (87). Also, organoids recapitulate many epigenomic features, which allow more accurate prediction of in vivo genome editing outcome (88).

After a thorough sequencing of the target site and genome-wide sequencing of off-target sites, the selected editing agent could be delivered as DNA, mRNA, or RNP by choosing from various vehicles, including AAV, lentivirus, eVLP, lipid nanoparticles, gold nanoparticles, and others (Fig. 3). The custom-designed treatment would then be administered to the selected patients.

While it is our hope that all patients with any form of IRD mutation could be treated, the implementation of precision genome editing faces a realistic economic challenge. The widespread use of rAAV for gene therapy continues to be limited by many factors, including scalability of manufacturing methods, expense of the clinical trials and testing, rarity of the disease, and governmental regulation. Also, the price varies across different countries depending on government, payor, and general public pricing. Nevertheless, Luxturna, which is the same formulation for all patients with *RPE65* mutations, was introduced with a heavy price tag at $425,000 per eye. This potential cost barrier raises questions about the pricing and affordability of the anticipated wave of personalized gene editing therapies, considering the resources that would need to be invested to develop unique gene editing therapies for individualized use. To make precision genome editing feasible as a clinically effective approach, development of economic models for commercializing therapies for rare disease should take place simultaneously with the clinical trials.

Conclusion

Precision medicine for IRDs has a promising outlook, as basic science has consistently led to the development of therapeutic tools to target patient-specific genetic mutations. In particular, advances in the design and delivery of BEs and PEs have improved their efficiency, safety, and versatility. However, most preclinical studies have been conducted in rodent models of ocular diseases, and translation from animal models to human eyes remains challenging. Efficient administration of therapeutic vectors to sufficient numbers of target cells is one of the most important objectives for successful therapy. In addition, development of a minimally invasive delivery procedure at the RPE layer is critical for prevention of retinal detachment and leakage of vectors into the vitreous fluid. The results of initial clinical trials that use in vivo gene editing to treat IRDs will be essential for informing the design and translation of future precision genome editing therapies.

Data, Materials, and Software Availability. All study data are included in the article and/or supporting information. There are no data underlying this work.

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