Current landscape of gene-editing technology in biomedicine: Applications, advantages, challenges, and perspectives

Weilin Zhou1,# | Jinrong Yang1,2,# | Yalan Zhang1 | Xiaoyi Hu1,3 | Wei Wang1,*

1Department of Biotherapy, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, People’s Republic of China
2Department of Hematology, Hematology Research Laboratory, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, P. R. China
3Department of Gynecology and Obstetrics, Development and Related Disease of Women and Children Key Laboratory of Sichuan Province, Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, West China Second Hospital, Sichuan University, Chengdu, P. R. China

*Correspondence
Wei Wang, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu 610041, People’s Republic of China.
Email: weiwang@scu.edu.cn

Abstract
The expanding genome editing toolbox has revolutionized life science research ranging from the bench to the bedside. These “molecular scissors” have offered us unprecedented abilities to manipulate nucleic acid sequences precisely in living cells from diverse species. Continued advances in genome editing exponentially broaden our knowledge of human genetics, epigenetics, molecular biology, and pathology. Currently, gene editing-mediated therapies have led to impressive responses in patients with hematological diseases, including sickle cell disease and thalassemia. With the discovery of more efficient, precise and sophisticated gene-editing tools, more therapeutic gene-editing approaches will enter the clinic to treat various diseases, such as acquired immunodeficiency syndrome (AIDS), hematologic malignancies, and even severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. These initial successes have spurred the further innovation and development of gene-editing technology. In this review, we will introduce the architecture and mechanism of the current gene-editing tools, including clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated nuclease-based tools and other protein-based DNA targeting systems, and we summarize the meaningful applications of diverse technologies in preclinical studies, focusing on the establishment of disease models and diagnostic techniques. Finally, we
1 | INTRODUCTION

An enormous amount of genetic information is hidden in the nucleotide sequence; sometimes, only a single base mutation has the potential to cause incurable diseases and even death. Over the past decade, the rapid expansion of gene-editing technology has constantly reshaped our conception of human genetics. These exquisite molecular tools have driven a massive revolution in many industries, especially the therapeutic industry. The “genomic scalpel” offers a profound opportunity to investigate genetic information, expanding our understanding of gene functions. Continued efforts to understand and elucidate these disease-causing mutations will be crucial to improving health care for patients with genetic disorders. Previous preclinical studies have demonstrated that the pathological process can be reversed using gene-editing molecules to inactivate or correct morbid mutations. These promising results inspired the efforts to develop gene-editing therapeutics to treat human genetic diseases in the clinic. In addition to genetic disorders, the scope of gene-editing applications has been extended to other conditions, including cancer and viral infections. Scientists in both biomedical research and commercial exploitation have displayed great enthusiasm for therapeutic gene editing. However, to reach the ultimate goal in which all genetic disorders can be cured by personalized gene editing, many difficult problems still need to be addressed. For instance, clinical applications require safer and more accurate engineered enzymes and more efficient delivery approaches. Regulating this technology to avoid abuse is also a focus of public concern.

In this review, we outline the development of gene-editing technology and introduce the most commonly used gene-editing tools. Next, we describe the current state of genome editing applications in fundamental research, focusing on developing disease models and diagnostic techniques. Finally, we summarize the clinical application of therapeutic gene editing and highlight the opportunities and challenges.

2 | CONSTANTLY UPDATED GENOME-EDITING TECHNOLOGY

In the 1980s, restriction endonuclease from bacteria was used to cleave the DNA double helix. Since then, scientists have made a great effort to enrich the genome-editing arsenal to manipulate gene sequences at specific loci. Different classes of genome manipulation tools are currently exploited for multiple applications in laboratory investigation, agriculture, and medicine. Common tools include zinc finger nucleases (ZFNs), transcription activator-like (TAL) effector nucleases (TALENs), clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) protein, base editors, and primer editors. It is clear that every gene-editing method has different mechanisms and operational constraints. However, most genetic modifications from various tools (when not extended to base and primer editors) rely on generating a double-strand break (DSB), which will trigger the endogenous repair mechanism in eukaryotic cells. Two endogenous DNA repair pathways within mammalian cells are generally responsible for repairing DSBs, including nonhomologous end-joining (NHEJ)-mediated repair and homology-directed repair (HDR; Figure 1). Theoretically, any gene segment can be incorporated into the genome by providing a homologous template via the HDR pathway. Given that NHEJ is typically dominant in most mammalian cells, improving the efficiency of HDR is crucial to maximizing the power of gene-editing platforms. Therefore, knowing the mechanism and characteristics of diverse genome-editing technologies, along with choosing suitable gene-editing agents, contributes to reaching our aspiration of performing genetic change precisely (various gene-editing technologies have been summarized in Table 1).

2.1 | CRISPR-Cas nucleases: A robust and versatile genome-editing tool

In nature, the various CRISPR-Cas systems discovered in bacteria and archaea are adaptive immune mechanisms
FIGURE 1  Overview of DNA damage repair mechanisms. DNA damage repair systems can be classified into two main groups: DNA double-strand break (DSB) repair and single-strand break (SSB) repair. Generally, DSBs are repaired by nonhomologous end-joining (NHEJ) or homology-directed repair (HDR). SSBs are repaired by nucleotide excision repair (NER) or base excision repair (BER). In the NHEJ pathway, DNA damage can be recognized by the Ku70/Ku80 complex and repaired by subsequent nucleases and ligases. The HDR pathway can achieve effective lesion repair using template-directed DNA. The NER contains two signaling pathways, global genomic NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER used the heterotrimeric lesion recognition factor (consisting of XPC, RAD23, and CETN2) to detect DNA lesions, but TC-NER used RNA polymerase II (RNAPII), Cockayne syndrome A (CSA) and CSB protein. The BER pathway is responsible for resolving nonbulky single-base lesions. Gene editing agents use these pathways to achieve genetic modification that silence foreign nucleic acids to resist invasion by the pathogen. In brief, once Cas effectors assemble with guide RNA molecules containing spacers, the complex can bind and cleave specific sequences near the protospacer adjacent motif (PAM). This mechanism inspired researchers to reprogram the DNA-recognizing capability of the CRISPR-Cas system by using different nucleases and guide RNA. Generally, CRISPR-Cas systems are classified into two groups (six types and more than 20 subtypes) according to their components and mechanisms. For
### TABLE 1  Comparison between different gene-editing agents

| Gene-editing technology | Clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas; CRISPR-Cas) system | Base editors | Primer editors | Zinc finger nucleases (ZFNs) | Transcription activator-like effector nucleases (TALENs) |
|------------------------|----------------------------------------------------------|--------------|----------------|-------------------------------|--------------------------------------------------------|
| Component for sequence recognition | Single guide (sgRNA) or CRISPR RNAs (crRNA)/ trans-activating crRNA | sgRNA | sgRNA | Zinc finger proteins | Transcription activator-like effectors (TALE) |
| Element for nucleic acid cleavage | Cas endonucleases (e.g.: SpCas9, Cas12a) | None | Cas9 nickase domain | FokI endonucleases | FokI endonucleases |
| Restrictions of target site | Adjacent to protospacer-adjacent motifs (PAMs) | Adjacent to PAMs, ssDNA R-loop | Adjacent to PAMs | Avoid non-G rich sites | TALEN monomer located 5’-end only recognizes T |
| length of target site | About 23 bp | > 17 bp | About 30 bp | (9–18 bp)*2 | (12–20 bp)*2 |
| Size | Cas nuclease > 2 kb, ~5.3 kb | ~6.4 kb | ~1 kb*2 | ~3 kb*2 |
| Advantages | Simple design and preparation, low cytotoxicity, high specificity, multiple gene editing | Reduce off-target effects, single base editing without double-strand breaks and homology-directed repair | Multiple edit capability: single base editing, insertion and deletion of multiple bases | Large recognition range, moderate editing efficiency | Easy to design, high specificity |
| Disadvantages | Potential off-target toxicity and on target mutation, low editing efficiency | The restriction of PAM, less efficiency | Potential indels mutation, less efficiency, immunogenicity | Cytotoxicity, potential off-target toxicity, inefficient cutting capability | Cytotoxicity, complex assembly, high production cost |

*Note: SpCas9, CRISPR-Cas9 from *Streptococcus pyogenes*.\n
instance, class 1 CRISPR-Cas systems, including Type I, Type III, and Type IV, can cleave nucleotide sequences depending on multiprotein complexes. In contrast, class 2 systems, which are subdivided into Type II, Type V, and Type VI, use single Cas effectors to achieve DNA cleavage. Because of their unique programmability and structural advantage, class 2 systems have been widely used in multiple genome-editing applications. Among the class 2 CRISPR-Cas systems, Cas9, Cas12, and Cas13 are currently the research focus.

2.1.1 CRISPR-Cas9 system

The natural CRISPR-Cas9 systems are Type-II CRISPR systems and are composed of DNA endonucleases and two RNA modules. A blunt DSB could be generated by this ribonucleoprotein (RNP) complex (Figure 2): CRISPR RNAs (crRNAs) paired with trans-activating crRNAs (tracrRNAs) guide nucleases to target specific loci and facilitate complex formation; then, endonucleases are responsible for cutting nucleic acids. To optimize their operability, the crRNA and tracrRNA were integrated into a single guide RNA (sgRNA).

The characteristics of various Cas9 systems, including the PAM specificity, spacer length, endonuclease activity, and guide RNA architecture, directly determine the precise recognition and cleavage process. For example, CRISPR Cas9 from Streptococcus pyogenes (SpCas9) was the first reported Cas9 variant that was tested for manipulating genetic sequences in vitro and in mammalian cells. For SpCas9 to perform normal functions, it is required for the user to choose an appropriate target location adjacent to the PAM 5′-NGG (N represents any nucleotide) and design a 20 nt spacer within the sgRNA or crRNA/tracrRNA pair as well as an active nuclease. In practice, scientists have discovered and developed many Cas9 variants to expand the scope of gene-editing applications. These Cas9 orthologs have shown diverse characteristics from SpCas9, such as smaller protein size (Staphylococcus aureus Cas9 contains 1053 amino acids, Campylobacter jejuni Cas9 contains 984 amino acids) and abundant PAM sequences that broaden the target scope (S. thermophilus Cas9 recognizes the PAM 5′-NNAGAAW, and W represents A or T).

2.1.2 CRISPR-Cas12 nuclease

CRISPR-Cas12 effectors from the Type-V CRISPR system are also RNA-guided endonucleases. Several innate features distinguish Cas12 from CRISPR-Cas9 systems (Figure 2). For instance, the recognition site of most Cas12 systems is located downstream of the PAM sequence, and the cleavage site within the protospacer is farther from the PAM. In addition, many Cas12 variants usually produce a staggered nucleic acid incision with a 4–5 nt overhang downstream of the PAM sequence by using a single crRNA. The first engineered Cas12 nuclelease named Cas12a (formerly Cpf1) has been applied to modify the genome information in human cells. The Cas12a effector inherently possesses the capacity to generate self-required crRNA from crRNA arrays without needing tracrRNA. This unique advantage means that this system has the potential to simplify multiplexed gene editing since scientists simply need to design multiple crRNA arrays to achieve this goal. Within Cas12a family proteins, two orthologs from Acidaminococcus spp. (AsCpf1) and Lachnospiraceae spp. (LbCpf1) have been shown to display gene-editing activity in human cells by recognizing the T-rich PAM (5′-TTTV). In addition to targeting and cutting DNA, some Cas12 variants (such as Cas12 g) can cleave the RNA when guided by a single crRNA. The discovery of Cas12-family endonucleases has propelled technical innovation in genome editing.

2.1.3 CRISPR-Cas13

After a series of bioinformatic analyses and computational efforts using microbial metagenomic database, some CRISPR-Cas13 variants were identified by Feng Zhang and colleagues, including Cas13a (formerly named C2c2), Cas13b, Cas13c, and Cas13d. Similar to Cas9 or Cas12 systems, Cas13 systems possess programmable nuclease activity and have the potential to be developed as RNA editing tools. The Cas13a effectors from the class 2 Type VI system are RNA-guided RNA endonucleases that have been optimized and show activity in manipulating RNA (Figure 2). Recently, specific orthologs of Cas13, including Cas13b and Cas13d, were leveraged for RNA knockdown and manipulation and exhibited stability and high efficiency in RNA editing. In addition to RNA knockdown and editing, Cas13 systems have been reformulated for nucleic acid detection. The Cas13 protein will cleave any single-stranded RNA in its vicinity after binding and cutting the targeted sequence. Based on the collateral cleavage activity of Cas13-family endonucleases, Zhang et al. established a diagnostic platform termed specific high-sensitivity enzymatic reporter unlocking (SHERLOCK). Compared with the traditional nucleic acid detection method quantitative polymerase chain reaction (qPCR), this diagnostic technology can detect RNA or DNA within 10–15 min with great sensitivity.
FIGURE 2  General overview of the primary gene-editing tools. Schematic diagram of various gene-editing tools. (A) Three frequently used clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) nucleases: Cas9, Cas12a, and Cas13a. (B) Cytosine base editors (CBEs) are composed of the Cas9 nickase (Cas9n), a cytidine deaminase, and a uracil glycosylase inhibitor (UGI). Adenine base editors (ABEs) are composed of the Cas9n and engineered adenosine deaminase. (C) The prime editor consists of Cas nickase and reverse transcriptase. (D) and (E). Two protein-based DNA targeting agents. Zinc finger nucleases (ZFNs) contain a zinc finger motif and the FokI restriction endonuclease. Transcription activator-like effector nucleases (TALENs) have catalytic FokI endonucleases fused to a DNA-binding domain TALENs.

more Cas13 orthologs have been discovered and tested for other applications, such as transcriptome regulation and RNA visualization in live cells.54

2.2  Base editors: The pearl in the crown of gene editing

Modest gene mutations, even a single nucleotide variation, may trigger the occurrence of genetic disease in humans.55 Currently, many genome-editing tools have been applied to correct genetic mutations. Of these cases, most strategies must generate DSBs and depend on the HDR pathway. However, common single base modification, which depends on the DNA repair pathway induced by DSBs, is far from satisfactory.56,57

To solve these issues, two different types of base editors based on CRISPR-Cas have been developed for precise base manipulation. One is cytosine base editors (CBEs), and the other is adenine base editors (ABEs).58 These tools were able to install point mutations without DSB through the single-strand repair pathway (Figure 1).59
2.2.1 CBEs install C•G-to-T•A mutations

The basic components of CBEs include a personalized sgRNA and a multifunctional fusion protein that consists of catalytically deficient CRISPR-Cas nuclease and single-stranded DNA cytidine deaminase (Figure 2). According to guidance by sgRNA, Cas effectors can bind the target sequence and create an ssDNA R-loop without generating DSBs. Generally, the editing window is a short fragment of exposed ssDNA (positions 4–8 are located in the protospacer if positions 21–23 are PAM). The cytidine deaminases are able to complete the transition point mutations (cytosines are converted to uracils) on the noncomplementary strand.

To date, scientists have created multiple versions of CBEs to gain greater efficiency and safety. The first-generation base editor, termed BE1, was formed using the cytidine deaminase enzyme APOBEC1 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-1) from *Rattus norvegicus* and a dead Cas9 (dCas9) without endonuclease activity. However, the mismatched base pair (U•G) is rectified through the base excision repair pathway, which may result in poor BE1 editing effectiveness in mammalian cells. To boost the base editing efficiency, the second-generation base editor BE2 was developed by adding an extra uracil glycosylase inhibitor (UGI). Because of the UGI, the U•G intermediate could remain in vivo longer to obtain stable base editing outcomes. To further increase the editing efficiency, the third version of the base editor (BE3, also described as CBE) replaced dCas9 with Cas9 nickase (Cas9n). Once the nondeaminated strand has been cleaved, the activated cellular repair mechanism will use the U-containing DNA strand as a template for fixing the gap. Additionally, many variants of BE3 have been engineered to improve the editing precision by narrowing the editing window, namely, variants such as YE1-BE3, YE2-BE3, and YEE-BE3. Most recently, the fourth-generation base editor BE4, which possesses two UGI proteins, was created for superior editing outcomes.

2.2.2 ABEs install A•T-to-G•C mutations

The initial design of ABEs was a Cas9n tethered to an adenosine deaminase (ADA). As originally conceived, ABE can deaminate adenine within the R-loop into inosine, which was eventually restored to guanine. However, the natural ADA cannot convert adenosine within ssDNA to inosine. Therefore, researchers have tried to reform the tRNA-specific ADA (ecTadA) from *Escherichia coli* using directed evolution. After complex protein engineering, the desired mutation was discovered and fused to Cas9n. The final architecture of ABEs was composed of a heterodimeric ADA (ecTadA-Tad*) and a Cas9n. This version was named ABE7.10, and it is the most commonly used base-editing tool. The discovery of ABE7.10 offers the possibility of extending the range of base editing. Similar to CBEs, ABEs have been continuously upgraded to pursue superior editing effectiveness. For instance, ABE-max with several nuclear localization signal peptides has been developed based on ABE7.10.

2.3 Prime editors: A potent new base modification technology

Four transition point mutations (A→G, T→C, C→T, and G→A) have been implemented by using CBEs and ABEs without reliance on the HDR pathway. However, the implementation of base transversions and the other eight types of point mutations (A→C, A→T, T→A, T→G, C→A, C→G, G→C, and G→T) require novel technology. In 2019, Liu's lab reported on a pioneering gene-editing tool called prime editor, which could not only install all possible types of DNA point mutations but could also induce small deletions or insertions without donor DNA templates. The current prime editor consists of a prime editing guide RNA (pegRNA) and a Cas9n fused to engineered reverse transcriptase (Figure 2). The protein complex guided by pegRNA nicks the target DNA strand and mediates subsequent reverse transcriptional reactions. In fact, the pegRNA is a modified sgRNA that has an extra RNA sequence at the 3′ end. This guide RNA containing the desired gene fragment is responsible for recognizing the target sequence and serves as a template for subsequent reverse transcription. In brief, the DNA single-strand nicked by Cas9n could use the pegRNA as the template to execute reverse transcription. After the reaction, the mutations are placed at the target site, generating a 5′ DNA flap and a 3′ DNA flap containing the edited sequence. Last, the 5′ DNA flaps were generally removed by the cellular DNA repair pathway. Compared with other genome-editing tools, prime editors have advanced in some respects, including a more extensive application range, as well as better editing accuracy and safety with fewer byproducts. The superprecise CRISPR tool enhances our ability to manipulate genetic information and has the potential to promote the development of gene therapy.

2.4 ZFNs: The first generation of mature gene-editing technology

ZFNs, which consist of zinc finger motifs and the FokI restriction endonuclease, are considered one of the
earliest gene-editing tools (Figure 2). Zinc finger proteins are responsible for recognizing and binding nucleotide sequences and are versatile transcription factors in eukaryotic organisms.\(^{73}\) Each zinc finger protein consists of approximately 30 amino acids and forms a \(\beta\beta\alpha\) structure, with a Cys2-His2 domain interacting with the zinc atom.\(^{74}\) According to the three-dimensional cocrystal structure, the DNA recognition capacity of zinc finger proteins (ZFs) relies on the \(\alpha\)-helical reading head bound with 3 bp within the major groove of the double helix.\(^{75}\) The Type II restriction enzyme FokI derived from Flavobacterium okeanokoites requires dimerization to complete DNA cleavage, and the cutting ability is independent without side effects.\(^{76,77}\) Generally, dimerized ZFNs can recognize 18–36 bps of the target sequence and generate a staggered DSB with a 5′ overhang. In principle, ZFNs can target any desired gene sequence by using various zinc finger proteins. In addition, the DNA binding specificity also depends on the number of fingers and the target location. However, the production of highly specific ZFNs is far from satisfactory since the assembly and optimization procedure required a great deal of time and effort. To extend the range of DNA recognition, many strategies, such as “two-finger modules”\(^{78}\) and the “open system,”\(^{79}\) have been established to reform and optimize the structure and function of ZFNs. For example, scientists obtained new ZFN architectures with a larger recognition range by using new linkers derived from a cleavage-based bacterial selection system.\(^{80}\)

Many advantages, including low immunogenicity and appropriate gene size, have prompted the widespread use of ZFNs as gene-editing tools for multiple applications. Unfortunately, the further large-scale application and popularization of ZFNs were hindered by some technical barriers. Admittedly, the synthesis and assembly of zinc finger proteins are time-consuming and require technical expertise. In addition, the related technology patents belong to several agencies, which hinders the widespread use of ZFNs.

## 2.5  TALENs: A flexible protein-based editing system

Because of the shortage of publicly available resources, it is difficult to construct ZFNs. To overcome these technical limitations, a protein-based DNA editing platform known as TALENs was established. TALENs contain a catalytic FokI endonuclease fused to DNA-binding domain TALEs (Figure 2). Natural TAL effectors have been discovered in plant pathogenic Xanthomonas bacteria, and they change the transcription in the host cells.\(^{81}\) The DNA targeting capacity of TALEs depends on highly conserved tandem arrays, which consist of 10–30 repeats. Individual TALE repeats that contain approximately 33–35 amino acids could target a single nucleotide accurately. The architecture of a single TALE includes two helix bundles and a loop. Notably, two hypervariable residues (residues 12 and 13) within the loop structure, which is currently named the repeat variable di-residue (RVD), determine the specificity and affinity of each effector.\(^{82}\) The RVD can not only ensure the stability of the loop structure but can also target specific nucleotides.\(^{83}\) To date, four identified RVDs, HD (HD represents the residues of histidine and aspartic acid, specifies cytosine), NG (NG represents the asparagine and glycine residues, specifies thymine), NI (NG represents the asparagine and isoleucine residues, specifies adenine), and NN (NN means two asparagine residues, specifies guanine or adenine), have been widely employed to generate the desired TALENs for multiple gene-editing applications.

Similar to ZFNs, the platform based on TALENs can be reprogrammed to bind arbitrary sequences by rearranging TALE repeats, but the complicated assembly procedure limits its promotion. To accelerate the assembly process, several strategies have been implemented, including the Golden Gate cloning system, restriction enzyme and ligation technology, fast ligation-based automatable solid-phase high-throughput system, and ligation-independent cloning technology.\(^{84}\) In addition to their programmability, TALENs have several obvious advantages: simplified design and construction and a flexible range of recognition. For instance, dimerized TALENs are usually engineered to bind 36-bp sequences or even longer. Of course, there are some disadvantages that must be solved. The large size of TALENs (approximately 3 kilobases [kb]) may cause delivery difficulties. During the delivery of TALENs, the repeats are susceptible to rearrangements,\(^{85}\) which carries a risk of genetic manipulation.

## 3  GENOME EDITING FOR FUNDAMENTAL RESEARCH: DISEASE MODELLING AND DISEASE DIAGNOSIS

### 3.1  Targeted genome editing tools for disease modeling

The unprecedented ability to create a gene modification at a specific site has driven the development and upgrading of medical disease models. Numerous successful disease models have been established for further detailed pathologic studies.

#### 3.1.1  Cardiovascular disease (CVD) models

CVD is considered a critical public health issue because of its high mortality and morbidity.\(^{86}\) Most CVDs are
inherited diseases characterized by monogenic mutations or a complex of heterozygous mutations. Carroll et al. created a Cas9 transgenic mouse model that only expresses a high level of Cas9 in the heart, but no evident burden was observed in vivo. Driven by adeno-associated virus (AAV), sgRNA was delivered to the Myh4 locus to induce an accurate and rapid depletion of cardio-relevant genes and subsequently show severe cardiomyopathy. The fibrillin-1 encoded by the FBN1 gene is the fundamental component of the connective tissue matrix. Heterozygous mutations in FBN1 often correlate with the phenotype of Marfan syndrome (MFS). Using pigs as a human biomedicine model could promote the study of human diseases. Many medical studies require a suitable animal model that resembles humans to repeat experiments. For example, the porcine model is considered a more suitable candidate for investigating CVD because of its unique biological characteristics. Specifically, the anatomical, physiological, and genetic characteristics of pigs are similar to those of humans. Some porcine somatic cells have been modified by ZFNs to generate a cloned pig with a heterozygous FBN1 mutant (+/Glu433AsnfsX98), which exhibited phenotypes (scoliosis, pectus excavatum, and structural damage in the aortic medial tissue) similar to those of patients with MFS. Based on a similar strategy, Yang et al. took advantage of ZFNs and electroporation to knock out the gene encoding peroxisome proliferator-activated receptor-gamma (Ppar-γ) in primary porcine cells and then generated an invaluable Ppar-γ knockout (KO) porcine model for studying CVD.

### 3.1.2 Metabolic disease models

Metabolic disorders represent a dysfunctional state in the body, hampering the shifting of food energy into cell bioreactions. Leptin (Lep) and its corresponding receptor (LepR) are responsible for inhibiting fat synthesis and balancing energy metabolism. To elucidate their pivotal function in glycolipid metabolism, scientists have created multiple animal models, which are typically represented by obesity mutant mouse models and diabetes mutant mouse models. Using CRISPR-Cas9 technology, Bao and colleagues established LepR knockout rats, which ultimately exhibited complications of obesity and diabetes. Furthermore, Chen et al. successfully installed diverse LepR mutations into mouse embryos by TALENs, generating several transgenic rats. Obesity, insulin resistance, and metabolic disorders were observed in some rats with a frame-shifted or premature stop codon mutation. The study reported the first rat models for obesity research based on the Sprague Dawley strain. As the key regulator in the metabolism of glucose and lipids, cAMP (cyclic adenosine monophosphate)-responsive element-binding protein 3-like 3 (CREB3L3) is predominantly expressed in the liver and intestine. To investigate the different functions of CREB3L3 within various tissues, some Japanese scholars invented liver- and small intestine-specific CREB3L3 knockout mice through the CRISPR-Cas9 system. With the help of these mouse models, these investigators provided a new understanding regarding the role of CREB3L3 in different metabolic pathways.

### 3.1.3 Models for neurodegenerative diseases (NDs)

NDs (such as Huntington’s disease [HD], Alzheimer’s disease [AD] and Parkinson’s disease [PD]) have always been a threat to human health. Due to the deficiency in effective diagnostic and therapeutic schemes, the public health care system suffers from stress. In particular, the dearth of suitable disease models has hampered progress in the field. Currently, many in vitro and in vivo studies have demonstrated that the huntingtin (HTT) gene is closely related to the occurrence of HD. To elucidate the pathogenesis of HD, diverse animal models have been investigated through gene editing. After introducing truncated mutant HTT into HD140Q knock-in mice through the CRISPR-Cas system, investigators found that exon 1 HTT is a key pathogenic factor of HD. Furthermore, Yan et al. generated a gene-engineered porcine model with full-length mutant HTT using CRISPR-Cas9 and somatic nuclear transfer technology. These large animal models recapitulated overt and selective neurodegeneration exhibited in HD patients, while pathological features can be germline-transmitted to their progeny. The amyloid precursor protein (APP) gene has also been extensively studied in NDs, specifically AD. To obtain APP-overexpressing cell lines, the partial coding regions of APP were incorporated into the genome of mouse fibroblast cells by ZFNs. These modified cells illustrate how APP mutants influence relevant signaling pathways to induce the onset of AD. TALENs were also used to create A673 V and A673T variants based on human-induced pluripotent stem cells (iPSCs), which ultimately developed various levels of AD-featured biomarkers. In addition, Paquet et al. installed both heterozygous and homozygous mutations of APP or presenilin (PSEN) 1 in human iPSCs enabling the appearance of early AD symptoms.

### 3.1.4 Hereditary ocular diseases

In recent years, emerging gene-editing technology has made it possible to establish hereditary eye disease models,
which help to explain the pathological mechanisms and to identify pathogenic genes. The existing eye disease models are primarily concentrated on retinitis pigmentosa (RP), Leber congenital amaurosis (LCA), and retinoblastoma, among others.

Receptor expression enhancer protein 6 (REEP6) is highly expressed in rod photoreceptor cells, contributing to the formation of the endoplasmic reticulum. Several biallelic variants of REEP6 have been identified in seven unrelated individuals affected with RP. Therefore, one of the verified missense mutants, which is known as REEP6.I, was integrated into the murine genome through CRISPR-Cas9, inducing clinical manifestations of RP, such as retinal degeneration and the malfunction of rod photoreceptors. LCA, a congenital eye disease, can lead to retinopathy and early blindness. At present, numerous mouse models have been generated to support the functional exploration of all other LCA genes. For instance, some evidence has shown that KCNJ13 mutants are closely linked to the early onset of LCA. Subsequently, different concentrations of spCas9RNA and sgRNA were delivered into zygotes using microinjection to generate a KCNJ13 gene-null mouse model. The results show that high concentration-derived mice are more efficient and compatible with mimicking the pathogenesis of KCNJ13-induced LCA and that the deficiency of KCNJ13 causes photoreceptor degeneration.

3.1.5 Other diseases

Von Willebrand disease (VWD) is the most common bleeding disorder and is characterized by coagulation disorders. Hai et al. reported a convenient and efficient method for preparing VWD models. They took advantage of the CRISPR-Cas9 platform to knock out the double allele of the VWF gene in pigs. This miniature pig model perfectly circumvents the current obstacle in which small rodents cannot highly recapitulate the disease hallmarks of human patients. Rett syndrome (RTT) is a serious disorder affecting children’s neurodevelopment. Arrested growth predominantly occurs in females aged 6–18 months old. However, for males, RTT is fatal during the fetal period. Methyl-CpG binding protein 2 (MECP2) is an X-linked gene, the mutations of which correlate with the rise of the autism spectrum. A research team from China employed TALENs to create the first MECP2 mutation model based on rhesus and cynomolgus macaques. These models have shown almost identical embryonic lethality with human boys suffering from RTT. Additionally, when using the same gene-editing system, this team generated another cynomolgus monkey model with mutagenesis of the RTT, which displayed stark abnormalities in physiology, behavior, and body structure.

Thanks to the rapid advancement of genome-editing technologies, numerous severe and refractory diseases also have available disease models for study, such as sickle cell disease (SCD), Niemann–Pick disease, and Duchenne muscular dystrophy. Progress in the genetic engineering area will further extend the application of animal models to elucidate the more sophisticated molecular mechanisms and raise entirely new prospects for therapeutic modalities.

3.2 New disease diagnostic tools based on the CRISPR-Cas system

In many cases, efficient therapeutic intervention relies on the timely and accurate diagnosis of disease. The currently relevant application of gene editing has shown unique advantages in the diagnosis of diseases, especially cancer and viral infections. The currently reported CRISPR-Cas systems can be broadly divided into two classes and some subtypes, according to the nature of the effector protein complex. Of these systems, the Class 2 CRISPR system, including Types II, V, and VI, is principally applied to develop novel diagnostic tools. For instance, one group made an attempt to create a CRISPR-Cas9n-mediated strand displacement amplification method (abbreviated as CRISDA). Multiple experimental results indicate that CRISDA has the potential to be a powerful diagnostic tool because of its single-nucleotide specificity and versatility.

One interesting application of the CRISPR-Cas system is in the diagnosis of cancer. Cancer, as an important disease threatening human health, has always had a very high mortality rate. The important reason for mortality is that patients often miss the best time for treatment because of the shortage of early diagnostic approaches. The efficient early diagnosis of cancer is critical to making an effective intervention for disease progression and providing a better quality of life. In 2017, Gootenberg et al. proposed a brand-new concept using CRISPR-Cas13n-mediated diagnostic techniques, which offers another ultrasensitivity and specificity option for testing RNA and DNA sequences. The platform, SHERLOCK, offers a chance to screen disease-causing mutations. Using this method, researchers found that cancer-related EGFR-L858R (EGFR, epidermal growth factor receptor) and BRAF-V600E mutations with allelic fractions as low as 0.1% can be detected in cell-free DNA fragments. In subsequent studies, scientists successfully detected EGFR-L858R and EGFR-T790 M mutations in DNA fragments derived from non-small cell lung cancer (NSCLC) patients. Inspired by SHERLOCK, investigators used Cas12a and recombinase
polymerase amplification to establish another diagnostic tool named DNA endonuclease-targeted CRISPR trans reporter (DETECTR), which has attomolar sensitivity and detects infectious viruses associated with cancer. Typically, human papillomavirus (HPV) 16 and HPV18, the two most pro-oncogenic HPV species, can be identified and distinguished by DETECTR. In practical applications, the detection efficiency is satisfactory, and the detection rate fluctuates between 90% and 100%. Furthermore, a reusable electrochemical biosensor consisting of the CRISPR-Cas13a system and a catalytic hairpin DNA circuit has been leveraged to screen tumor-related RNA for the early diagnosis of NSCLC. Another interesting application of the CRISPR-Cas system is in the diagnosis of infectious diseases. Most currently, global health care systems have been undermined to an unprecedented extent by the novel coronavirus (COVID-19) pandemic, which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Accurate and rapid diagnosis will benefit patients to receive timely treatment. However, enzyme-linked immunosorbent assay (ELISA) and real-time or reverse transcription-polymerase chain reaction (RT-PCR) are common and classical molecular detection tools that require intricate equipment and fastidious work processes. It is imperative to establish a portable, accurate, and convenient diagnostic system for detecting infectious disease pathogens. Diagnostic CRISPR systems have outstanding superiority in terms of their ultrahigh sensitivity, portability, and specificity. For instance, the Cas13 and Cas13 nucleases have been reprogrammed to detect the nucleic acids of SARS-CoV-2.

Viruses, whether DNA viruses or RNA viruses, are important pathogens of infectious diseases. RNA viruses that have been studied abundantly in recent years include dengue, Zika, and a recent spotlight of interest, SARS-CoV-2. For SARS-CoV-2 detection, the SHERLOCK and DETECTR detection systems mentioned above have been modified and upgraded to achieve higher efficiency in virus extraction, more flexibility in sensing capability, and a more streamlined mechanical process. In 2022, Lu et al. reduced the detection time of CRISPR-based assays while ensuring accuracy through a hybrid strategy, which includes adjusting the kinetics of Cas12a and using more flexible crRNA designs.

In addition to RNA viruses, CRISPR/Cas-based diagnostic methods can identify DNA viruses, such as BK virus (BKV), cytomegalovirus (CMV), and Epstein–Barr virus (EBV). The researchers used the SHERLOCK system to detect BKV or CMV in serum samples and then verified the results by qPCR. The comparison results demonstrated that the two approaches have almost the same specificity. To date, the Food and Drug Administration (FDA) has authorized a CRISPR-based COVID-19 diagnostic tool for emergency use. The successful translational application of the CRISPR-Cas system in COVID-19 diagnosis indicated that this versatile technology has the potential to be applied to other severe epidemic disease diagnoses in the future.

4. CURRENT APPLICATION OF GENE EDITING TO THE TREATMENT OF HUMAN DISEASES

The ultimate aspiration pursued by researchers is to use gene-editing technologies to treat human diseases without undesired side effects. The current gene-editing techniques have provided a brilliant opportunity for precise intracellular gene manipulation, which can not only be used to induce mutations, corrections, or deletions but can also introduce foreign genes at specific sites. Additionally, precise genetic manipulation can efficaciously reduce the risk of insertion mutations in some cell therapies. Admittedly, novel therapies offer an attractive opportunity to save patients’ lives. In contrast, the consequence of gene editing is unknown and fraught with potential risks. Therefore, performing sufficient preclinical research is an important prerequisite for promoting gene editing for clinical application. As described in the previous section, advances in disease modeling and diagnostic techniques have driven the development of preclinical research on gene-editing therapeutics. According to these unique advantages and the promising outcomes of preclinical studies, various gene-editing agents have been leveraged to treat human diseases in clinical trials.

Generally, the strategies of gene-editing therapeutics contain two modes, including in vivo and ex vivo strategies (Figure 3). To date, numerous pre- and clinical studies of gene editing have been scattered across various countries. In this section, we will provide a comprehensive overview of this clinical information and primarily discuss the progress in therapeutic gene editing in clinical trials (relevant clinical trial information is summarized in Table 2). Additionally, we also introduce some interesting preclinical studies and highlight several current novel therapeutic strategies and concepts.

4.1 Cancer immunotherapy

There are currently more than 70 clinical trials in the NIH clinical trial database that involve gene editing-mediated therapy, including the use of ZFNs, TALENs, or CRISPR/Cas, and nearly 50% of these trials are related to neoplasms. Cancer immunotherapy is widely considered one of the most significant advances in biological
FIGURE 3  Ex vivo and in vivo therapeutic gene-editing strategies. Gene-editing therapeutics consist of two modes. The in vivo gene-editing strategy (left) is straightforward. The vectors containing desired gene cargoes and editing machinery are injected into the targeted tissues or organs to perform gene editing. The treatment process of ex vivo gene-editing therapy (right) can be roughly divided into the following four steps: (1) separate the required cells from the donor and culture them in vitro; (2) use an appropriate gene-editing platform to modify the cell genome; (3) expand and cultivate the edited cells in vitro; and (4) inject the edited cells back into the patient for treatment.

AAV, adeno-associated virus; TALENs, transcription activator-like effector nucleases; ZFNs, zinc finger nucleases
| Therapeutic area | Disease application | Edited cells | Target | Number of included patients | Platform | Delivery | Clinical phase | Identifier | Status |
|------------------|---------------------|--------------|--------|----------------------------|----------|----------|---------------|------------|--------|
| Cancer           | B-cell acute lymphoblastic leukemia (B-ALL) | T cells | TRAC and CD52 | 25 | TALEN | mRNA electroporation | I | NCT02746952 | Completed |
|                  | B-ALL | T cells | TRAC and CD52 | 60 | TALEN | mRNA electroporation | I | NCT04150497 | Recruiting |
|                  | Relapsed/refractory large B-cell lymphoma | T cells | TRAC and CD52 | 74 | TALEN | mRNA electroporation | I | NCT03939026 | Active, not recruiting |
|                  | Relapsed/refractory follicular lymphoma | T cells | Endogenous inactivate hematopoietic progenitor kinase 1 | 40 | CRISPR | mRNA electroporation | I | NCT04037566 | Recruiting |
|                  | ALL in relapse | T cells |  |  |  |  |  |  |  |
|                  | ALL in refractory | T cells |  |  |  |  |  |  |  |
|                  | B-cell lymphoma | T cells |  |  |  |  |  |  |  |
| B-cell malignancy | Non-Hodgkin lymphoma | T cells | β2M and T-cell receptor (TCR) | 143 | CRISPR-Cas9 | Electroporation | I/II | NCT04035434 | Recruiting |
|                  | B-cell lymphoma | T cells | β2M and TCR | 80 | CRISPR-Cas9 | Electroporation | I/II | NCT03166878 | Recruiting |
|                  | B-cell leukemia | T cells | TRAC | 80 | CRISPR-Cas9 | Electroporation | I/II | NCT03398967 | Recruiting |
|                  | B-cell lymphoma | T cells | TRAC and PDCD1 | 50 | chRdNA | Plasmid transfection | I | NCT04637763 | Recruiting |
|                  | B-ALL | T-cell | TRAC and CD52 | 10 | CRISPR-Cas9 | Lentiviral vector | I | NCT04557436 | Recruiting |
|                  | B-cell non-Hodgkin lymphoma | T cells | PD-1 | 50 | CRISPR | / | I | NCT04637763 | Recruiting |
|                  | Relapsed/refractory B-ALL | T cells | CD52 and TRAC | 10 | CRISPR | / | I | NCT04925206 | Recruiting |
|                  | T-cell lymphoma | T cells | β2M and TCR | 45 | CRISPR-Cas9 | Electroporation | I | NCT04382446 | Recruiting |
|                  | T-cell acute lymphoblastic leukemia | T cells | CD7 | 21 | CRISPR-Cas9 | / | I | NCT03690011 | Recruiting |
|                  | T-cell acute lymphoblastic lymphoma | T cells |  |  |  |  |  |  |  |
|                  | T-non-Hodgkin Lymphoma | T cells |  |  |  |  |  |  |  |

(Continues)
| Therapeutic area | Disease application | Edited cells | Target | Number of included patients | Platform | Delivery | Clinical phase | Identifier | Status |
|------------------|---------------------|--------------|--------|-----------------------------|----------|----------|----------------|------------|--------|
| Relapsed/refractory CD5⁺ hematopoietic malignancies | T cells | CD5 | 18 | CRISPR-Cas9 | Electroporation | I | NCT04767308 | Not yet recruiting |
| Acute myeloid leukemia | T cells | TRAC and CD52 | / | TALEN | mRNA electroporation | I | NCT04106076 | Withdrawn |
| Relapsed/refractory acute myeloid leukemia | T cells | TRAC and CD52 | 65 | TALEN | mRNA electroporation | I | NCT03190278 | Recruiting |
| Acute myeloid leukemia | T cells | TRAC and CD52 | 54 | CRISPR-Cas9 | mRNA electroporation | I/II | NCT05066165 | Recruiting |
| Relapsed/refractory multiple myeloma (MM) | T cells | TRAC and CD52 | 18 | TALEN | mRNA electroporation | I | NCT0442619 | Recruiting |
| Acute myeloid leukemia | Hematopoietic stem and progenitor cell | CD33 | 33 | CRISPR-Cas9 | / | long-term follow-up (LTFU) study | NCT04426656 | Recruiting |
| MM | T cells | β2M and TCR | 80 | CRISPR-Cas9 | Electroporation | I | NCT04244656 | Recruiting |
| MM | T cells | TCR PDCD1 | 3 | CRISPR | mRNA electroporation | I | NCT03399448 | Terminated (Sponsor has terminated trial to pursue other targets.) |
| Melanoma synovial sarcoma | T cells | TCR PDCD1 | 3 | CRISPR | mRNA electroporation | I | NCT04244656 | Recruiting |
| Myxoid/round cell liposarcoma | T cells | PDCD1 | 12 | CRISPR-Cas9 | Plasmid Electroporation | I | NCT02793856 | Completed |
| Metastatic non-small cell lung cancer | T cells | PDCD1 | 20 | CRISPR-Cas9 | Plasmid | I/II | NCT00262669 | Recruiting |

(Continues)
| Therapeutic area | Disease application | Edited cells | Target | Number of included patients | Platform | Delivery | Clinical phase | Identifier | Status |
|------------------|---------------------|--------------|--------|-----------------------------|----------|----------|----------------|------------|--------|
| Solid tumor      | T cells             | PDCD1        | 10     | CRISPR-Cas9                 | Protein and mRNA electroporation | I        | NCT03747965   | Unknown    |
| Mesothelin-positive multiple solid tumors | T cells | TCR PDCD1 | 10 | CRISPR-Cas9 | Protein and mRNA electroporation | I | NCT03545815 | Recruiting |
| Renal cell carcinoma | T cells | β2M and TCR | 107 | CRISPR-Cas9 | Electroporation | I | NCT04438083 | Recruiting |
| Esophageal cancer | T cells | PDCD1 | 16 | CRISPR-Cas9 | Electroporation | NA | NCT03081715 | Completed |
| Neurofibromatosis type 1 tumors of the central nervous system | Induced pluripotent stem cells (iPSCs) | NF1 | 20 | CRISPR-Cas9 | DNA | NA | NCT03332030 | Suspended (Suspended due to cessation of funding) |
| Advanced hepatocellular carcinoma | T cells | PDCD1 | 10 | CRISPR-Cas9 | DNA | I | NCT04417764 | Recruiting |
| Invasive bladder cancer stage IV | T cells | PDCD1 | Withdrawn | CRISPR-Cas9 | DNA | I | NCT02863913 | Withdrawn |
| Metastatic renal cell carcinoma | T cells | PDCD1 | Withdrawn | CRISPR-Cas9 | DNA | I | NCT02867332 | Withdrawn |
| Metastatic renal cell carcinoma | T cells | PDCD1 | Withdrawn | CRISPR-Cas9 | DNA | I | NCT02867332 | Withdrawn |
| Stage IV gastric carcinoma | T cells | PDCD1 | 20 | CRISPR-Cas9 | Electroporation | I/II | NCT03044743 | Unknown |
| Stage IV nasopharyngeal carcinoma | Cytotoxic T lymphocyte cells | TGF-β receptor II | 30 | CRISPR-Cas9 | / | I | NCT04976218 | Recruiting |

(Continues)
| Therapeutic area | Disease application | Edited cells | Target | Number of included patients | Platform | Delivery | Clinical phase | Identifier | Status |
|------------------|---------------------|--------------|--------|-----------------------------|----------|---------|----------------|------------|--------|
| Hematological diseases | Hemophilia B | Hepatocytes | Clotting factor XI gene | 1 | ZFN | Adeno-associated virus (AAV) | I | NCT02695160 | Terminated |
| | Sickle cell disease (SCD) | Autologous hematopoietic stem cells (HSCs) | BCL11A | 8 | ZFN | mRNA | I/II | NCT03653247 | Recruiting |
| | Transfusion-dependent beta-thalassemia | Autologous CD34+ hematopoietic stem/progenitor cells | BCL11A | 6 | ZFN | mRNA | I/II | NCT03432364 | Active, not recruiting |
| | Transfusion-dependent beta-thalassemia | Autologous CD34+ hematopoietic stem/progenitor cells | BCL11A | 6 | ZFN | mRNA | I/II | NCT03432364 | Active, not recruiting |
| | Thalassemia | iHSCs | HBB gene | 12 | CRISPR-Cas9 | / | Early I | NCT03728322 | Unknown |
| Beta-thalassemia | CD34+ human hematopoietic stem and progenitor cells | BCL11A gene | 45 | CRISPR-Cas9 | Electroporation | I/II | NCT03655678 | Active, not recruiting |
| Inborn hematologic diseases | CD34+ human hematopoietic stem and progenitor cells | BCL11A gene | 8 | CRISPR-Cas9 | Electroporation | I | NCT04925206 | Recruiting |
| | Transfusion dependent beta-thalassemia | CD34+ human hematopoietic stem | BCL11A gene | 8 | CRISPR-Cas9 | Electroporation | I | NCT04925206 | Recruiting |
| | SCD | CD34+ human hematopoietic stem and progenitor cells | BCL11A | 45 | CRISPR-Cas9 | Electroporation | I/II | NCT03745287 | Active, not recruiting |
| | SCD | CD34+ human hematopoietic stem and progenitor cells | BCL11A | 15 | CRISPR-Cas9 | Adenoviral vector | I/II | NCT04819841 | Recruiting |
| | SCD | CD34+ human hematopoietic stem | β-globin gene | 9 | CRISPR-Cas9 | Electroporation | I/II | NCT04774536 | Not yet recruiting |
| | Transfusion dependent β-thalassemia | CD34+ Human Hematopoietic Stem and Progenitor Cells (hHSPCs) | BCL11A | 8 | CRISPR-Cas9 | Electroporation | I | NCT04925206 | Recruiting |

(Continues)
| Therapeutic area | Disease application | Edited cells | Target | Number of included patients | Platform | Delivery | Clinical phase | Identifier | Status |
|------------------|---------------------|--------------|--------|-----------------------------|----------|----------|---------------|------------|--------|
| Severe SCD       | CD34+ hHSPCs        | BCL11A gene  | 12     | CRISPR-Cas9                 |          | /        | III           | NCT05329649 | Not yet recruiting |
| Severe SCD       | CD34+ HSCs          |              | 15     | CRISPR-Cas9                 |          | /        | I/II          | NCT04819841 | Recruiting |
| Viral infections | Human immunodeficiency virus (HIV) infection | Autologous CD4+ T cells | C-C chemokine receptor type 5 (CCR5) | 12 | ZFN | Adenoviral vector | I | NCT00842634 | Completed |
| HIV infection    | Autologous CD4+ T cells | CCR5        | 21     | ZFN                         | Adenoviral vector | I/II      | NCT01252641 | Completed |
| HIV infection    | Autologous CD4+ T cells | CCR5        | 19     | ZFN                         | Adenoviral vector | I          | NCT01044654 | Completed |
| HIV infection    | Autologous CD4+ T cells | CCR5        | 8      | ZFN (SB-728mR)              | Electroporation | I/II      | NCT02225665 | Completed |
| HIV infection    | Autologous CD4+ T cells | CCR5        | 14     | ZFN (SB-728mR)              | Electroporation | I          | NCT02388594 | Completed |
| HIV infection    | HSPCs               | CCR5        | 12     | ZFN                         | Electroporation | I          | NCT02500849 | Active, not recruiting |
| HIV infection    | T cells             | CCR5        | 12     | ZFN                         | mRNA      | I          | NCT03617198 | Active, not recruiting |
| HIV infection    | T cells             | CCR5        | 30     | ZFN                         | Adenoviral vector | I/II      | NCT03666871 | Active, not recruiting |
| HIV infection    | HIV infected cells and tissues | Remove viral DNA from the genomes of cells and tissues | 9 | CRISPR-Cas9 | Adenovirus-associated virus vector serotype 9 (AAV9) | I | NCT05144386 | Recruiting |
| HIV infection    | HIV infected cells and tissues | Remove viral DNA from the genomes of cells and tissues | 9 | CRISPR-Cas9 | AAV9 | LTFU study | NCT05143307 | Enrolling by invitation |
| Human papillomavirus (HPV)-related malignant neoplasm | Tumor cells | HPV16 E6/E7 | 40 | TALEN | Roche plasmid transfection | I | NCT03226470 | Recruiting |

(Continues)
| Therapeutic area | Disease application | Edited cells | Target | Number of included patients | Platform | Delivery | Clinical phase | Identifier | Status |
|------------------|---------------------|--------------|--------|-----------------------------|----------|----------|----------------|------------|--------|
| HPV-related malignant neoplasm | HPV16 E6/E7 Tumor cells | HPV16 E6/E7 | 60 | TALEN and CRISPR-Cas9 | Plasmid transfection | I | NCT03057912 | Unknown |
| HPV-related malignant neoplasm | HPV16 E6/E7 Tumor cells | HPV16 E6/E7 | 20 | ZFN | Plasmid transfection | I | NCT02800369 | Unknown |
| Coronavirus disease (COVID-19) | PD-1and ACE2 T cells | PD-1and ACE2 | 16 | CRISPR-Cas9 | / | I/I | NCT04990557 | Not yet recruiting |
| Refractory herpetic viral keratitis | Herpes simplex virus type I Herpessimplex virus type I | Herpes simplex virus type I | 6 | CRISPR-Cas9 | / | I/I | NCT04560790 | Active, not recruiting |

| Metabolic disorders | Mucopolysaccharidosis II | Hepatocytes | IDS gene | 9 | ZFN | AAV | I/I | NCT03041324 | Terminated |
|---------------------|---------------------------|--------------|----------|----|------|------|------|------------|------------|
| Adenosine deaminase (ADA) deficiency severe combined immunodeficiencies | Autologous HSPCs | ADA gene | 36 | / | Lentivirus | I/I | NCT01380990 | Completed |
| Type 1 diabetes mellitus | Pancreatic endoderm cells | | 10 | CRISPR-Cas9 | / | I | NCT05210510 | Recruiting |
| Transthyretin (TTR)-related (ATTR) familial amyloid polyneuropathy | Hepatocytes | TTR protein gene | 74 | CRISPR-Cas9 | Lipid nanoparticles (LNPs) | I | NCT04601051 | Recruiting |
| Transthyretin-related (ATTR) familial amyloid cardiomyopathy | | | | | | | | |
| Wild-type transthyretin cardiac amyloidosis | | | | | | | | |

| Ocular disorder | Blindness Leber congenital amaurosis 10 vision disorders | Photoreceptor cells | CEP290 | 18 | CRISPR-Cas9 | AAV | I/I | NCT03872479 | Recruiting |

| Others | Kabuki syndrome 1 | Mesenchymal stem cells | KMT2D | 8 | CRISPR-Cas9 | / | NA | NCT03855631 | Completed |
|---------|------------------|-------------------------|--------|----|----------------|----|-----|------------|------------|
|         | Rubinstein–Taybi syndrome | iPSCs | CREBBP | 154 | CRISPR-Cas9 | / | NA | NCT04122742 | Recruiting |
|         | Hereditary angioedema | Hepatocytes | KLKB1 | 55 | CRISPR-Cas9 | LNPs | I/I | NCT05120830 | Recruiting |

**Note:** All data in Table 2 were collected from ClinicalTrials.gov, which is a resource provided by the US National Library of Medicine (https://clinicaltrials.gov).
has already initiated clinical trials for treating B-cell lymphoma (NCT04416984). Another company, CRISPR Therapeutics, has invented three UCAR-T therapies based on the CRISPR gene-editing system, namely, CTX110, CTX120, and CTX130. Unlike Cellectis Inc., CRISPR Therapeutics chooses to destroy β2M and TCR loci to reduce the risk of rejection. CTX110 is a CD19-specific CAR-T that is primarily used to treat B-cell malignancy (NCT04035434). CTX120 kills MM cells by recognizing BCMA (NCT04244656), while CTX130 is used to treat CD70-expressing T-cell lymphoma and renal cell carcinoma (NCT04502446; NCT04438083). In addition, some researchers designed “off the shelf” CAR-T cells against CD19 or mesothelin to treat B-cell hematological or solid tumors (NCT03166878; NCT03545815).

Apart from UCAR-T therapy, gene modification technology can be used to disrupt endogenous genes to improve the effectiveness of CAR-T cells. For example, in a Phase I clinical trial, researchers used the CRISPR system to inactivate hematopoietic progenitor kinase 1, an intracellular negative regulator of T-cell proliferation and signal transduction, to enhance the effect of CD19 CAR-T cells (NCT04035756). In another Phase I clinical trial conducted in the Chinese PLA General Hospital (NCT04976218), CAR-T cells that targeted EGFR were edited by CRISPR-Cas9 to knock out transforming growth factor beta (TGF-β), which is considered a major regulatory factor in the immunosuppressive tumor microenvironment (TME), and CAR-EGFR-TGFβR-KO T cells may relieve the hostile TME in solids and improve the treatment effect. Antigen loss is the main reason for the failure of tumor immunotherapy; one of the strategies is to target more than one target simultaneously. A clinical trial conducted by the Chinese PLA General Hospital evaluated the feasibility and safety of universal bispecific CD19+CD20+ and CD19+CD22+ CAR-T cells for treating relapsed or refractory B-cell leukemia and lymphoma (NCT03398967).152

The well-known immune checkpoints PD-1 and CTLA-4 can significantly inhibit the activity of T cells. Tumor cells can easily escape the immune response by virtue of this mechanism, resulting in poor therapeutic effects. Using gene-editing tools to destroy endogenous immune checkpoint genes to enhance the antitumor effect is a novel treatment strategy that is widely used in the treatment of solid tumors. In 2016, the first clinical trial of PD-1 knockout T cells for treating NSCLC was launched at West China Hospital of Sichuan University (NCT02793856). Investigators took advantage of CRISPR-Cas9 to edit the PD-1 gene of T cells ex vivo. After culturing and expanding in vitro, the T cells were reinfused into the subject, which supported the safety and feasibility of this therapy in NSCLC for the first time.153 Additionally, in a CRISPR-related clinical trial led by Professor Carl June, researchers eliminated the genes encoding PD-1 and endogenous TCR through CRISPR-Cas9, effectively enhancing the effect of CAR-T cells targeting melanoma (NCT03399448). At present, PD-1 knockout T cells have also been used for treating advanced hepatocellular carcinoma (NCT04417764), invasive bladder cancer (NCT02863913), metastatic renal cell carcinoma (NCT02867332), esophageal cancer (NCT03081715), and EBV-associated malignancies (NCT03044743), elevating the progress in cancer immunotherapy.154–157

In addition to adoptive cell transfer therapy, many novel therapeutic strategies have been tested for treating tumors in preclinical studies. For example, the success of the SARS-CoV-2 mRNA vaccine depended on the advanced lipid nanoparticle (LNP) delivery system.158,159 This efficient delivery vehicle has the potential to perform therapeutic gene editing in vivo. In a preclinical study, novel amino-ionizable LNPs encapsulating Cas9 mRNA and gRNAs were injected into orthotopic glioblastoma to disrupt the polo-like kinase 1 gene. In animal models, this strategy had positive and safe therapeutic outcomes.160 Gao et al. constructed a new Cas13a expression vector containing a nuclear factor κB (NF-κB)-specific promoter and U6 promoter. The expression of Cas13a is controlled by NF-κB, which is widely overactivated in various cancers. Once the promoter is activated, the expression of endogenous oncogenes can be regulated by designing different sgRNAs within the vector.161 Furthermore, prime editors and some Cas variants circumvent specific adverse effects in traditional genome editing and catalyze the editing process without the requirement for DSBs.162

4.2 Infectious diseases caused by viruses

Genome editing is expected to become a powerful tool in antiviral therapy that acts by modifying infection-related genes required for viral invasion and replication in host cells. Through gene editing, virus-resistant immune or stem/progenitor cells can be produced, which could prevent or alleviate viral diseases.163 C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4), the main auxiliary receptors for human immunodeficiency virus (HIV), play major roles in the initial infection and the establishment of stable infection, respectively. Additionally, homozygous carriers of the CCR5 Delta 32 mutation are naturally resistant to HIV infection, suggesting that the artificial deletion of CCR5 may be used to endow T cells with the feature of HIV infection resistance.164 Previous studies have used different genome editing tools to inactivate the CCR5 gene and CXCR4 gene on CD4+ T cells and CD34+
hematopoietic stem and progenitor cells (HSPCs) successfully,\textsuperscript{165–167} and these findings showed that these cells are not susceptible to HIV.\textsuperscript{168,169} Among anti-HIV therapies, the strategy of deleting the CCR5 gene through ZFN is relatively mature, and many clinical trials have been approved and undertaken. The first clinical trial using gene editing to treat HIV was led by Carl June in 2009. Researchers used ZFNs (SB-728) to inactivate the CCR5 gene of autologous CD4+ T cells and reinfused these genetically modified T cells (SB-728-T) into 12 recruited patients. The results showed that except for one patient who exhibited serious adverse transfusion reactions, the remaining patients were tolerant to genetically engineered T cells,\textsuperscript{170} indicating that CCR5-modified autologous T-cell infusion is safe and feasible (NCT00842634). Subsequently, a plurality of clinical trials focused on determining the therapeutic dose and impact of SB-728-T to establish an efficacious clinical protocol (NCT03666871, NCT01044654, and NCT01252641). With the continued emphasis on the safety and effectiveness of immunotherapy, novel treatment strategies have emerged, including transfecting CCR5-specific ZFN mRNA to edit T cells (NCT02225665, NCT02388594) or knocking out the CCR5 gene in CAR-T cells to treat HIV (NCT03617198). Scientists wished to explore the effects of genetically modified T cells on HIV infection resistance under these strategies. In addition to genetically modifying T cells, scientists would like to modify hematopoietic stem or progenitor cells to create a cell pool in vivo that can continue to produce T cells resistant to HIV infection. In a clinical study, researchers from the City of Hope Medical Center delivered CCR5-specific ZFN mRNA into HSPCs to evaluate its safety in patients infected with HIV-1 (NCT02500849).\textsuperscript{171} Moreover, in a recent clinical study, Chinese scientists designed a stable CRISPR-Cas9 system to edit the CCR5 gene of donor-derived CD34+ hematopoietic stem cells (HSCs) and infused these cells back into the patient.\textsuperscript{102} They then evaluated the feasibility and safety of this therapeutic strategy in patients infected with HIV. After patients with ALL and HIV infections received the infusion of these autologous CCR5-deficient HSPCs, the acute lymphocytic leukemia was in complete remission, and CCR5-deficient donor cells remained in the body for more than 19 months. This case indicated that in humans, CCR5-inactivated HSPCs contributed to long-term hematopoietic system reconstruction.\textsuperscript{172} However, the low efficiency of gene editing limits the therapeutic effect, suggesting that the focus of this research may be to improve the editing efficiency. The above cases have supported the immense potential of gene-editing technology in treating HIV and put forward further requirements for the efficiency of gene editing and the optimization of the treatment scheme.

Another strategy for antiviral therapy based on gene manipulation is to target the viral genome associated with viral replication and assembly directly. In addition to addressing HIV infection, gene-editing platforms have been applied to other causative agents, including HPV, hepatitis B virus (HBV), and EBV. The main etiological factors of cervical cancer are some specific oncoproteins. The HPV E6/E7 genes encode important oncoproteins related to the neoplastic transformation of the disease. Multiple clinical trials have attempted to introduce different gene-editing modules to target and destroy the DNA of HPV16/18 E6 or E7 directly in vivo. The researchers hope that the tumorigenetic process will be reversed in situ and that the incidence of cervical cancer will be reduced (NCT03226470, NCT03057912, and NCT02800369).\textsuperscript{173–175} A similar idea was adopted for the elimination of HBV. To inhibit HBV replication, researchers used lentivirus to deliver CRISPR-Cas9 nuclease and HBV-specific sgRNA into HepG2 cells bound to HBV.\textsuperscript{176}

The COVID-19 led the World Health Organization to declare a pandemic in March 2020, and it remains a significant threat to human health worldwide. Zhang et al. designed a novel diagnostic tool termed SHERLOCK to detect the nucleic acids of viruses.\textsuperscript{126} Inspired by the SHERLOCK system, American scientists transformed CasI3a into an antiviral agent that was programmed to detect and destroy RNA viruses in human cells in another novel study. The antiviral activity of CasI3a and its diagnostic ability were combined to construct a system that may be used to diagnose and treat viral infections, which is termed Carver (i.e., CasI3-assisted restriction of viral expression and readout).\textsuperscript{177}

## 4.3 Hematological diseases

Many hematological disorders are caused by genetic mutations, including thalassemia, hemophilia, and SCD. The correction of erroneous gene mutations can be accomplished by gene-editing technology, which undoubtedly holds promise for treating hereditary hematological diseases.

The \textit{HBB} gene mutation on chromosome 11 reduces \(\beta\) globin chain production, which in turn causes \(\beta\)-thalassemia.\textsuperscript{178} HSCs have significant advantages in reconstructing or restoring human hematopoietic function. A clinical experiment attempted to modify induced HSCs from patients to correct the mutated \textit{HBB} gene and then transfuse them back into the patients to restore their normal hemoglobin production ability (NCT03728322). The presence of fetal hemoglobin (HbF) can in some cases alleviate the symptoms of thalassemia, but HbF production is suppressed by BCL11A.\textsuperscript{179,180} Thus, knocking this gene out
or down leads to a suitable approach to treating SCD and beta-thalassemia. An experimental CRISPR-based therapy (known as CTX001) invented by CRISPR Therapeutics has undergone two clinical trials for treating SCD and thalassemia (NCT03655678; NCT03745287). The key strategy underlying CTX001 is to use CRISPR-Cas9 to modify the BCL11A gene within CD34+ human hematopoietic stem and progenitor cells to increase the production of HbF in vivo. Sangamo Therapeutics and Sanofi also conducted two clinical trials using a similar strategy. However, ZFNs were used instead of a CRISPR/Cas system for genetic modification (NCT03432364, NCT03653247). Furthermore, the appearance of base editors that have the capacity to manipulate a single base without DSBs provides an attractive option to cure sickle-cell disease. Liu et al. generated a bespoke base editor to convert the pathogenic gene (HBBS, hemoglobin subunit beta allele) into a nonpathogenic gene (HBBSG, Makassar β-globin). Based on this strategy, approximately 80% of hematopoietic stem and progenitor cells derived from patients could be engineered in vitro. Furthermore, humanized SCD mice that received edited HSPCs exhibited reduced splenic and hematologic pathology, compared to untreated mice. Hemophilia B is a blood clotting disorder caused by mutations in the clotting factor XI gene. In a Phase I clinical trial, researchers introduced ZFNs to patients intravenously. The editing agents could install the correct clotting factor XI gene into the albumin locus of hepatocytes. The aim of the clinical trial is to produce a permanent secretion of coagulation factor XI in the body (NCT02695160).

### 4.4 Metabolic disorders

Mucopolysaccharidosis is a metabolic disorder caused by the congenital absence of lysosomal enzymes. Furthermore, most mucopolysaccharidoses are autosomal recessive. This disease has seven typical clinical types, and two clinical trials for MPS I and MPS II are currently underway. MPS I is primarily due to a lack of α-L-iduronidase, while the shortage of iduronate sulfatase primarily causes MPS II. Angamo Therapeutics Inc. used AAV-derived vectors to deliver gene-editing components into hepatocytes. These ZFNs could insert a normal α-L-iduronidase gene or iduronate 2-sulfatase gene into albumin sites to obtain lifetime lysosomal enzyme production capacity (NCT02702115, NCT03041324). Additionally, several published reports have demonstrated the safety and effectiveness of the CRISPR system in treating MPS I.

ADA deficiency is a metabolic disease caused by mutations in the ADA gene. In a previous clinical study, the patient-derived HSPC genome was corrected by introducing ADA complementary DNA, and the corrected cells were then autologously transplanted into patients (NCT01809990). It is worth noting that the gene fragment was introduced by lentivirus, which may carry a risk of insertion mutation.

In Type 1 diabetes mellitus, the islet cells that produce insulin in the pancreas are mistakenly attacked by effector T cells, failing to respond to blood glucose changes. Additionally, regulatory T (Treg) cells cannot properly interfere with this false attack, which ultimately leads to the progression of diabetes. Researchers use gene-editing technology to target the forkhead box P3 (FOXP3) gene in human T cells, inserting a robust enhancer/promoter proximal to the first coding exon. The persistent activation of the FOXP3 gene allows T cells to be artificially transformed into Treg cells. These edited Treg cells have the potential to stop the negative reaction within the pancreas for treating diabetes. The clinical benefits of gene-editing therapeutics for Type 1 diabetes still require more study.

### 4.5 Neurodegenerative disorders

NDs are caused by the loss of neuronal structure or function in the brain and spinal cord, including AD, PD, HD, and amyotrophic lateral sclerosis (ALS). The number of patients gradually increases due to the lack of effective early diagnosis and successful therapeutic intervention. Additionally, as limited by the understanding of the pathogenesis of NDs, the exploitation of therapeutics is extremely challenging. Genetic mutation and the aggregation of misfolded proteins are considered potential pathogenic mechanisms of ND. Therefore, correcting gene or protein errors based on the gene-editing platform is promising for exploring and treating NDs. There are thousands of clinical trials on NDs. In addition to a few approved monoclonal antibodies, no ND treatments have displayed promising clinical outcomes. In addition, many results of clinical trials have yet to be verified. Currently, gene-editing therapeutics for NDs are still in their infancy. Thus, we primarily present some preclinical research progress here.

AD is one of the most common chronic NDs, and its primary pathological features are amyloid plaques and neurofibrillary tangles, which eventually lead to a severe cognitive disorder. Swedish scientists reported that they used the CRISPR-Cas9 system to correct the mutation of the gene encoding APP in a patient’s cells. This change could lead to a treatment for AD. In addition, scientists have generated several antibodies targeting β-amyloid protein for active or passive immunity. As significant components, apolipoprotein E and PSEN participate in the
disease process of AD and can serve as potential targets for the gene-editing system.192 HD is a rare ND with an autosomal dominant inheritance caused by an abnormality in the HTT gene.193 The accumulation of abnormal metabolites (the huntingtin protein) damages the cerebral cortex, leading to mental decline and the loss of athletic ability.194 Although tetraabenazine is approved for treating HD, the effect of the drug is limited. Current treatment strategies to alleviate HD primarily include directly replacing damaged or lost neurons, knocking out or silencing genes that express abnormal proteins, and reducing the harm caused by the huntingtin protein to protect the surviving neurons.195,196 The main pathological feature of PD involves the loss of dopaminergic neurons in the substantia nigra pars compacta and brain cell death.197,198 As there is no cure for PD, the current conventional treatment strategy is to compensate for the loss of dopamine artificially; however, this approach only relieves the symptoms. A recent study used the CRISPR-Cas9 system to inactivate the alpha synuclein (SNCA) gene encoding alpha-synuclein, which is related to the formation of Lewy bodies. Compared to unedited cells, edited stem cells can differentiate into dopamine-producing neurons and do not produce Lewy bodies when subjected to specific chemical stimuli. This work reaffirms the potential of gene editing-mediated cell replacement therapy in treating NDs.199,200 ALS is characterized by the extensive degeneration of motor neurons in the spinal cord, brain stem, and cerebral cortex.201,202 The current treatment strategy replaces damaged motor neurons with neural stem cells that secrete neurotrophic factors. Mutations in the gene encoding superoxide dismutase 1 are one of the pathological causes of ALS.203 Researchers from the University of California, Berkeley, verified that the deletion of the superoxide dismutase type 1 (SOD1) gene could improve the retention of motor neurons in a rat model.204,205

To conclude, the lack of feasible treatment options for NDs is temporary. Gene-editing platforms allow us to understand the occurrence of diseases at the genetic level and to establish more complete disease models.

4.6 Ocular disorder

The great potential of using limbal stem cells for treating corneal injury is described above. Gene therapy has also made significant progress in treating hereditary eye diseases. LCA is a major hereditary disease that causes blindness in children, of which LCA10 is the most common and the most serious type. In 2017, the US FDA approved a gene therapy called Luxturna (voretigene neparvovec-ryzl) to treat patients with LCA2 with RPE65 gene mutations.206–208 The pathological cause of LCA10 is a mutation of the CEP290 gene (the most common mutation is termed p.Cys998X).113 In 2020, Allergan and Editas Medicine Inc. initiated a clinical trial to treat LCA10 using AGN-151587 (EDIT-101), which could eliminate the mutation in CEP290 using Cas9 effectors, combined with the AAV vector (NCT03872479).

5 THERAPEUTIC GENOME EDITING: OPPORTUNITIES AND CHALLENGES COEXIST

5.1 Genome-editing technologies increase the potential to treat genetic diseases

According to the latest data from the OMIM Gene Map Statistics, more than 4000 genes will cause morbid phenotypes in humans.209 A total of millions of people worldwide suffer from genetic disorders. Unfortunately, most of them are unable to receive optimal treatments because of the dearth of therapeutic drugs and diagnostic methods.210 Traditional drug development to treat rare genetic diseases costs vast amounts of money and time, forcing many pharmaceutical companies to retreat. Exploiting a novel treatment for genetic diseases is urgently needed.211,212 The plight of unmet medical needs is currently expected to be addressed by therapeutic genome editing. Emerging gene-editing technologies have expanded our abilities to manipulate the gene sequences of eukaryotic cells.30,36,58,60,175 These molecular tools make it possible to cure genetic diseases by correcting or removing the errors within genome sequences.

Notably, therapeutic products based on gene-editing technologies have offered unique advantages over traditional small molecular drugs and antibodies.147 A prominent strength of gene therapy is that it can offer a customized therapeutic schedule for individuals who have limited or no treatment options.212 Of course, personalized clinical procedures may be restricted by regulatory guidelines and have a heavy financial burden. The promising clinical benefits, however, will encourage regulatory authorities to adjust their policies toward these emerging therapies.213 The treatment strategies for most gene-editing therapies can be briefly summarized in several steps, including screening, synthesis, delivery, the detection of target gene sequences and industrialized production.214 Compared to small-molecule targeted drugs and monoclonal antibodies, nucleic acid sequences are easy to design and less expensive.215 Additionally, the expanding gene-editing arsenal has provided many versatile tools to manipulate the gene sequence as needed.216 Last, the desired gene cargoes and editing tools could be
Several challenges limit the efficiency in developing clinical applications.

5.2.1 Efficiency

In addition to safety, the wide applications of genome editing depend on its efficiency. The common factors affecting editing efficiency include the following: (i) the target cell type and cellular environment, (ii) the optimal choice of gene-editing tools, (iii) the competition of different cellular DNA repair pathways, and (iv) the technologies for delivering editing components. Of these factors, the efficient delivery of genome editing machinery in vitro and in vivo is the foundation of successful gene-editing therapeutics. Therefore, we will focus on discussing the development of delivery strategies. Previous studies have developed multiple delivery methods to transfer macromolecules (such as proteins, DNA, siRNA, or mRNA) into the cell. The current delivery strategies can be classified into two formats: virus-based delivery systems and nonviral delivery systems (Figure 4).

A variety of viral vehicles, including AAVs, lentiviruses, adenoviruses, and retroviruses, have been applied to deliver gene-editing components into target cells of interest. Most currently, AAVs and lentiviruses, depending on some unique advantages, have been widely used in clinical trials. Natural AAV vectors have II serotypes, which possess inherent tropisms for different tissues. Because of the tropism for the eye, AAV2 has been approved by the FDA for treating degenerative retinal disorders. Moreover, AAV vectors could reduce the risk of genomic mutation because the gene cargos carried by AAV cannot integrate into the host genome. Although many attractive features of AAVs have been noted, some existing challenges are still awaiting address. The first challenge is the limited packing capacity of AAV vehicles. Most AAV vectors can encode up to 4.4 kb of exogenous DNA, less than other tools. Sometimes, the carrying capacity can accommodate only one CRISPR-Cas endonuclease gene without other space for sgRNA or donor templates. A feasible solution is using two vectors to deliver the whole system with the potential to reduce efficiency. The employment of smaller engineered Cas variants is an alternative strategy to tackle the capacity limit and improve efficiency. Second, the preexisting adaptive immunity to natural serotypes hinders the in vivo delivery effectiveness of AAV vectors. However, cap-
and in vivo delivery is impractical. Some investigators have tried to combine different delivery methods to improve editing efficiency. 264 Dai et al. established a system to generate CAR-T cells with immune checkpoint gene knockout using electroporation and AAV6 vectors. These attempts will accelerate the development of delivery systems. 265 In summary, even though current delivery systems have some disadvantages, emerging delivery strategies will offer more options for us to achieve ideal gene editing.

5.2.2 Safety considerations

However, as previously mentioned, an increasing number of clinical applications and tests have demonstrated the availability of gene editing. 153,266–268 There are still several technical limitations that hinder the broad clinical utility of genome editing. Scientists have made a great deal of effort to break through these limitations by developing various hybrid strategies.

The safety of therapeutic gene editing is a key concern for investigators and patients. 147,269,270 The primary security risks arise from the discrepant editing accuracy and immunogenicity of gene-editing effectors or delivery reagents. 271 Ideal editing outcomes involve the precise installation of desired mutations within the target site without creating any byproducts. However, due to deficient target specificity, DNA cleavage may occur at the wrong genome locations that have similar features to the intended editing sequence. In addition, the DSBs generated by various gene-editing nucleases generally induce different modes of DNA repair, including NHEJ and/or HDR. 272 In some cases, even if the desired DSBs appear in the target site, the unpredictable and complex repair process may also cause undesired mutagenesis. 221,273 For instance, once off-target events occur in cardiac cells,
even the frequency is small, which will cause irreversible problems.\textsuperscript{274} To improve the accuracy and precision of gene editing, some researchers have tried to generate new orthologs with greater specificity,\textsuperscript{275} such as SpCas9 and xCas9.\textsuperscript{25,276–278} Another approach is to avoid producing DSBs.\textsuperscript{70,279} Liu et al. demonstrated that base editors and prime editors could install a single base mutation or small fragment within genome sequences without reliance on DSBs and HDR.\textsuperscript{58,70} Moreover, it is worthwhile to establish effective methods for monitoring off-target events in humans.\textsuperscript{280} Some current evidence has suggested that in vitro analysis of off-target editing in primary cells can serve as a guide for in vivo situations.\textsuperscript{280}

The immunogenic toxicity of editing proteins is frequently mentioned in association with CRISPR-Cas systems. The engineered Cas nucleases were remodeled based on natural Cas proteins derived from some bacteria.\textsuperscript{10} If individuals have ever been infected by these pathogens, the engineered editing effectors have the potential to be captured by preexisting antibodies and trigger inflammation and other unknown side effects.\textsuperscript{281,282} Although some studies have detected specific antibodies and preexisting adaptive immunity to Cas9 in humans,\textsuperscript{282,283} we still need sufficient evidence to clarify the occurrence mechanism of immunotoxicity caused by preexisting antibodies.\textsuperscript{284} Together, these studies are helping to identify more secure proteins for gene editing in the clinic. The immune response to the delivery modality has been discussed in the previous section.

5.2.3 Ethical challenges

Many studies have highlighted the remarkable therapeutic benefits and promising future of gene-editing therapy. However, we cannot focus only on short-term successes and ignore the unique ethical challenges.\textsuperscript{285} Although germline changes have been completed in plants or some animals,\textsuperscript{286,287} there is some controversy over human germline alterations. There is no doubt that human germline genome editing must be rigorously regulated.\textsuperscript{288} In the absence of sufficient understanding, all scientists should stand in awe of human germline genome editing. Of course, some rational attempts should be allowed under strict supervision.

6 FUTURE OUTLOOK AND CONCLUSION

A profound revolution and innovation have been driven by gene-editing technologies in many fields, including agriculture, medicine, biotechnology, and the manufacturing industry. In the last decade, more efficient and versatile gene-editing platforms have been established and now offer us a powerful tool for investigation and genome engineering in eukaryotic cells. As a research tool, various gene-editing agents have enabled us to characterize and understand the functions of normal genes. In addition, scientists have the opportunity to screen disease-causing mutations and elucidate the pathogenesis of some rare genetic diseases. As a diagnostic tool, CRISPR-Cas systems have been re-engineered to detect viral nucleic acids, such as SARS-CoV-2. The successful application of gene editing in the diagnosis of infectious diseases indicates that CRISPR-Cas nucleases have the potential to be developed into an accurate and rapid diagnostic tool for other diseases. The most inspiring application of gene editing is in the field of gene or cell therapy. The correction and alteration of disease-causing gene mutations offer the possibility of treatment or even a permanent cure for some genetic disorders. Emerging gene manipulation tools have addressed many technological issues associated with immunotherapy. For instance, some immunotherapies, particularly CAR-T therapy, are poised to create a “paradigm shift” in malignant tumours. To date, we have witnessed many promising clinical outcomes and have accumulated increasing clinical experience.

Despite the fact that gene-editing therapeutics have been subject to tremendous progress in clinical applications, several formidable problems need to be approached before the ultimate aspiration of curing all genetic disorders can be fulfilled. First, scientists continue to increase the accuracy and efficiency of existing gene-editing agents in parallel with innovations and developments of novel technologies. Additionally, we still emphasize the urgent need for optimal delivery approaches, which are the major barriers to achieving efficient gene manipulation in vivo. Furthermore, bioethicists have stressed that the original intention of genome editing is to correct morbific errors rather than eliminate differences. Therefore, we should be vigilant about the deliberate or unintentional misuse of these customized tools.

Scientific technology offers a profound opportunity to reshape medical treatments. Realizing the full potential of gene-editing technology depends not only on the efforts of scientists and clinicians but also on the support of the government and other stakeholders. It is foreseeable that gene-editing technology will provide a novel avenue for health care in the future.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (81972878 and 82172733), the National Key Research and Development Program of China (2016YFC1303403 and 2020YFC0860200) and the
Key Research and Development Program of Sichuan Province (2020YFS0008).

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

ETHICS STATEMENT
The authors declare that human ethics approval was not needed for this study.

AUTHOR CONTRIBUTIONS
W.W. conceived and presented the article idea and supervised the whole work. W.Z. collected the data and wrote the first draft of the manuscript. J.Y., Y.Z., and X.H. participated in editing the manuscript. W.W. provided important suggestions for manuscript writing. All authors participated in the work and approved the manuscript for publication.

DATA AVAILABILITY STATEMENT
The data included in this study are available upon request from the corresponding author.

REFERENCES
1. Danna K, Nathans D. Specific cleavage of simian virus 40 DNA by restriction endonuclease of Hemophilus influenzae. Proc Natl Acad Sci U S A. 1971;68(12):2913-2917.
2. Broeders M, Herrero-Hernandez P, Ernst MPT, van der Ploeg AT, Pijnappel W. Sharpening the molecular scissors: advances in gene-editing technology. iScience. 2020;23(1):100789.
3. Gaj T, Gersbach CA. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397-405.
4. Rouet P, Smith F, Jasin M. Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. Proc Natl Acad Sci U S A. 1994;91(13):6064-6068.
5. O’Driscoll M, Jeggo PA. The role of double-strand break repair–insights from human genetics. Nat Rev Genet. 2006;7(1):45-54.
6. Lieber MR, Ma Y, Pannicke U, Schwarz K. Mechanism and regulation of human non-homologous DNA end-joining. Nat Rev Mol Cell Biol. 2003;4(9):712-720.
7. Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW. The double-strand-break repair model for recombination. Cell. 1983;33(1):25-35.
8. Chapman JR, Taylor MR, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. Mol Cell. 2012;47(4):497-510.
9. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem. 2010;79:181-211.
10. Lin S, Staahl BT, Alla RK, Doudna JA. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. Elife. 2013;2:e05766.
11. Hille F, Richter H, Wong SP, Bratovič M, Ressel S, Charpentier E. The biology of CRISPR-Cas: backward and forward. Cell. 2018;172(6):1259-1259.
12. Barrangou R, Fremeaux B, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315(5819):1709-1712.
13. Garneau JE, Dupuis M, Villion M, et al. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature. 2010;468(7320):67-71.
14. Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonuclease complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci U S A. 2012;109(39):E2579-2586.
15. Makarova KS, Wolf YI, Alkhnabashi OS, et al. An updated evolutionary classification of CRISPR-Cas systems. Nat Rev Microbiol. 2015;13(11):722-736.
16. Koonin EV, Makarova KS, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems. Curr Opin Microbiol. 2017;37:67-78.
17. Shmakov S, Abudayyeh OO, Makarova KS, et al. Discovery and functional characterization of diverse class 2 CRISPR-Cas systems. Mol Cell. 2015;60(3):385-397.
18. Makarova KS, Wolf YI,Iranzo J, et al. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. Nat Rev Microbiol. 2020;18(2):67-83.
19. Jiang F, Doudna JA. CRISPR-Cas9 structures and mechanisms. Annu Rev Biophys. 2017;46:505-529.
20. Kleinstiver BP, Prew MS, Tsai SQ, et al. Engineered CRISPR-Cas9 delivery. Trends Biotechnol. 2019;37:119-133.
21. Tycko J, Myer VE, Hsu PD. Methods for optimizing CRISPR-Cas9 genome editing specificity. Mol Cell. 2016;63(3):355-370.
22. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-821.
23. Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823-826.
24. Karvelis T, Gasiunas G, Young J, et al. Rapid characterization of CRISPR-Cas9 protospacer adjacent motif sequence elements. Genome Biol. 2015;16:253.
25. Hu JH, Miller SM, Geurts MH, et al. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature. 2018;556(7699):57-63.
26. Chen JS, Dagdas YS, Kleinstiver BP, et al. Enhanced proof-reading governs CRISPR-Cas9 targeting accuracy. Nature. 2017;550(7676):407-410.
27. Casini A, Olivier M, Petris G, et al. A highly specific SpCas9 variant is identified by in vivo screening in yeast. Nat Biotechnol. 2018;36(3):265-271.
28. Edraki A, Mir A, Ibraheim R, et al. A compact, high-accuracy Cas9 with a dinucleotide PAM for in vivo genome editing. Mol Cell. 2019;73(4):714-726.
29. Chatterjee P, Jakimo N, Jacobson JM. Minimal PAM CRISPR-Cas9 protospacer adjacent motif sequence elements. Trends Biotechnol. 2018;36(3):265-271.
30. Ran FA, Cong L, Yan WX, et al. In vivo genome editing using Staphylococcus aureus Cas9. Nature. 2015;520(7546):186-191.
31. Kim E, Koo T, Park SW, et al. In vivo genome editing with a small Cas9 orthologue derived from Campylobacter jejuni. Nat Commun. 2017;8:14500.
32. Yamada M, Watanabe Y, Gootenberg JS, et al. Crystal structure of the minimal Cas9 from Campylobacter jejuni reveals the molecular diversity in the CRISPR-Cas9 systems. Mol Cell. 2017;65(6):1109-1121. e1103.

33. Esvelt KM, Mali P, Braff JL, Moosburner M, Yang SJ, Church GM. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. Nat Methods. 2013;10(11):1116-1121.

34. Müller M, Lee CM, Gasiunas G, Davis TH, et al. CRISPReffectors. Nat Biotechnol. 2017;35(3):276-282.

35. Harrington LB, Burstein D, Chen JS, et al. Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. Science. 2018;362(6416):839-842.

36. Zetsche B, Gootenberg JS, Abudayeh OO, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;163(3):759-771.

37. Li T, Zhu L, Xiao B, Gong Z, Liao Q, Guo J. CRISPR-Cpf1-gRNA-guided endonuclease of a class 2 CRISPR-Cassystem. Cell Death Dis. 2017;8(6):1-14.

38. Zetsche B, Heidenreich M, Mohanraju P, et al. Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. Nat Biotechnol. 2017;35(1):31-34.

39. Kleinstiver BP, Sousa AA, Walton RT, et al. Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. Nat Biotechnol. 2019;37(3):276-282.

40. Gao L, Cox DBT, Yan WX, et al. Engineered Cpf1 variants with altered PAM specificities. Nat Biotechnol. 2017;35(8):789-792.

41. Kim D, Kim J, Hur JK, Been KW, Yoon SH, Kim JS. Genomewide analysis reveals specificities of Cpf1 endonucleases in human cells. Nat Biotechnol. 2016;34(8):863-868.

42. Yan WX, Hunnewell P, Alfonse LE, et al. Functionally diverse type V CRISPR-Cas systems. Science. 2019;363(6422):88-91.

43. Kim HK, Min S, Song M, et al. Deep learning improves prediction of CRISPR-Cpf1 guide RNA activity. Nat Biotechnol. 2018;36(3):239-241.

44. Cox DBT, Gootenberg JS, Abudayeh OO, et al. RNA editing with CRISPR-Cas13. Science. 2017;358(6366):1019-1027.

45. Abudayeh OO, Gootenberg JS, Esvelt KM, et al. RNA targeting with CRISPR-Cas13. Nature. 2017;550(6765):280-284.

46. Abudayeh OO, Gootenberg JS, Konermann S, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science. 2016;353(6299):aaaf5573.

47. Zhang C, Konermann S, Brideau NJ, et al. Structural basis for the RNA-guided ribonuclease activity of CRISPR-Cas13d. Cell. 2018;175(1):212-223. e217.

48. Konermann S, Lofy P, Brideau NJ, Oki J, Shokhirev MN, Hsu PD. Transcriprome engineering with RNA-Targeting type VI-D CRISPR effectors. Cell. 2018;173(3):665-676.

49. Gootenberg JS, Abudayeh OO, Lee JW, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017;356(6336):438-442.

50. Ai Y, Liang D, Wilusz JE. CRISPR/Cas13 effectors have differing extents of off-target effects that limit their utility in eukaryotic cells. Nucleic Acids Res. 2022.

51. Kellner MJ, Koob JG, Gootenberg JS, Abudayeh OO, Zhang F. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc. 2019;14(10):2986-3012.

52. Ackerman CM, Myhrvold C, Thakku SG, et al. Massively multiplexed nucleic acid detection with Cas13. Nature. 2020;582(7811):277-282.

53. Myhrvold C, Freije CA, Gootenberg JS, et al. Field-deployable viral diagnostics using CRISPR-Cas13. Science. 2018;360(6387):444-448.

54. Wang H, Nakamura M, Abbott TR, et al. CRISPR-mediated live imaging of genome editing and transcription. Science. 2019;365(6459):1301-1305.

55. Roth TL, Marson A. Genetic Disease and Therapy. Annu Rev Pathol. 2021;16:145-166.

56. Ihy RJ, Worringa KA, Salick MR, et al. p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat Med. 2018;24(7):939-946.

57. Komor AC, Badran AH, Liu DR. CRISPR-based technologies for the manipulation of eukaryotic genomes. Cell. 2017;168(1-2):20-36.

58. Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature. 2017;551(7681):464-471.

59. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533(7603):420-424.

60. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet. 2018;19(12):770-788.

61. Nishida K, Arazoe T, Yachie N, et al. Targeted nucleotide editing using hybrid protokaryotic and vertebrate adaptive immune systems. Science. 2016;353(6305).

62. Di Noia J, Neuberger MS. Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase. Nature. 2002;419(6902):43-48.

63. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. Nature. 1995;373(654):487-493.

64. Komor AC, Zhao KT, Packer MS, et al. Improved base excision repair inhibition and bacteriophage Mu Gam protein yields Cg-to-Ta base editors with higher efficiency and product purity. Sci Adv. 2017;3(8):eaaq4774.

65. Wang L, Xue W, Yan L, et al. Enhanced base editing by co-expression of free uracil DNA glycosylase inhibitor. Cell Res. 2017;27(10):1289-1292.

66. Kim YB, Komor AC, Levy JM, Packer MS, Zhao KT, Liu DR. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. Nat Biotechnol. 2017;35(4):371-376.

67. Liu Z, Chen S, Shan H, et al. Efficient base editing with high precision in rabbits using YFE-BE4max. Cell Death Dis. 2020;11(1):36.

68. Koblan LW, Doman JL, Wilson C, et al. Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. Nat Biotechnol. 2018;36(9):843-846.

69. Yu Y, Leete TC, Born DA, et al. Cytosine base editors with minimized unguided DNA and RNA off-target events and high on-target activity. Nat Commun. 2020;11(1):2052.

70. Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149-157.
71. Hegde ML, Hazra TK, Mitra S. Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res. 2008;18(1):27-47.

72. Ledford H. Super-precise new CRISPR tool could tackle a plethora of genetic diseases. Nature. 2019;574(7779):464-465.

73. Ugozov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet. 2010;11(9):636-646.

74. Pavletich NP, Pabo CO.锌finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1 A. Science. 1991;252(5007):809-817.

75. Li L, Wu LP, Chandrasegaran S. Functional domains in transcription factors. Cell Res. 1992;89(10):4275-4279.

76. Beerli RR, Barbas CF. Engineering polyzinc finger nucleases. Nat Rev Genom. 2001;982.20(2):135-141.

77. Doyle EL, Stoddard BL, Voytas DF, Bogdanove AJ. TAL effectors for studying neurodegenerative diseases. Proc Natl Acad Sci U S A. 2013;23(8):390-398.

78. Moore M, Klug A, Choo Y. Improved DNA binding specificity from polyzinc finger peptides by using strings of two-finger units. Proc Natl Acad Sci U S A. 2001;98(4):1437-1441.

79. Holkers M, Maggio I, Liu J, et al. Differential integrity of TALE nuclease genes following adenosine and lenti viral vector gene transfer into human cells. Nucleic Acids Res. 2013;41(5):e63.

80. Roth GA, Dwyer-Lindgren L, Bertozzi-Villa A, et al. Trends and patterns of geographic variation in cardiovascular mortality among US counties, 1980–2014. JAMA. 2017;317(19):1976-1992.

81. Zeng Y, Li J, Li G, et al. Correction of the Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991;352(6333):337-339.

82. Lunney JK, Van Goor A, Walker KE, Hailstock T, Franklin J, Dai C. Importance of the pig as a human biomedical model. Sci Transl Med. 2021;13(621):eabd5758.

83. Umezawa K, Watanabe K, Watanabe M, et al. Generation of heterozygous fibrillin-1 mutant cloned pigs from genome-edited foetal fibroblasts. Sci Rep. 2016;6(1):24413.

84. Yang D, Yang H, Li W, Zhao B, Ouyang Z, Liu Z, et al. Generation of FPARy mono-allelic knockout pigs via zinc-finger nucleases and nuclear transfer cloning. Cell Res. 2011;21(6):979-982.

85. O’Rahilly S. Human genetics illuminates the paths to metabolic disease. Nature. 2009;462(7271):307-314.

86. Coppari R, Bjorbek C. Leptin revisited: its mechanism of action and potential for treating diabetes. Nat Rev Drug Discov. 2012;11(9):692-708.

87. Giesbertz P, Padberg I, Rein D, et al. Metabolite profiling in plasma and tissues of ob/ob and db/db mice identifies novel markers of obesity and type 2 diabetes. Diabetologia. 2015;58(9):2133-2143.

88. Bao D, Ma Y, Zhang X, et al. Preliminary characterization of a leptin receptor knockout rat created by CRISPR/Cas9 system. Sci Rep. 2015;5:15942.

89. Chen Y, Lu W, Gao N, et al. Generation of obese rat model by transcription activator-like effector nucleases targeting the leptin receptor gene. Sci China Life Sci. 2017;60(2):152-157.

90. Tirronen A, Hokkanen K, Vuorio T, Ylä-Herttuala S. Recent advances in novel therapies for lipid disorders. Hum Mol Genet. 2019;28(1):R49-R54.

91. Nakagawa Y, Oikawa F, Mizuno S, et al. Hyperlipidemia and hepatitis in liver-specific CREB3L3 knockout mice generated using a one-step CRISPR/Cas9 system. Sci Rep. 2016;6:27857.

92. Lam S, Bayraktar A, Zhang C, et al. A systems biology approach for studying neurodegenerative diseases. Drug Discov Today. 2020;25(7):1146-1159.

93. Xu L, Yang H, Gao Y, et al. CRISPR/Cas9-mediated CCR5 ablation in human hematopoietic stem/progenitor cells confers HIV-1 resistance in vivo. Mol Ther. 2017;25(8):1782-1789.
109. Wang Y, Wu F, Pan H, et al. Lost region in amyloid precursor protein (APP) through TALEN-mediated genome editing alters mitochondrial morphology. Sci Rep. 2016;6:22244.

110. Paquet D, Kwart D, Chen A, et al. Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. Nature. 2016;533(7601):125-129.

111. Björk S, Hurt CM, Ho VK, Angelotti T. REPs are membrane shaping adapter proteins that modulate specific G protein-coupled receptor trafficking by affecting ER cargo capacity. PLoS ONE. 2013;8(10):e76366.

112. Arno G, Agraval Smriti A, Eblimit A, et al. Mutations in REPs cause autosomal-recessive retinitis pigmentosa. American Journal of Human Genetics. 2016;99(6):1305-1315.

113. den Hollander AI, Koene Koop RK, Yzer S, et al. Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. Am J Hum Genet. 2006;79(3):556-561.

114. Zhong H, Chen Y, Li Y, Chen R, Mardon G. CRISPR-engineered mosaicism rapidly reveals that loss of Rcnj13 function in mice mimics human disease phenotypes. Sci Rep. 2015;5:8366.

115. Denis CV, Susen S, Lenting PJ. Modeling Niemann-Pick disease type C1 in zebrafish: a robust platform for in vivo hematopoietic stem cells rescues sickle cell disease in mice. Am J Hum Genet. 2006;79(3):556-561.

116. Hai T, Teng F, Guo R, Li W, Zhou Q. One-step generation of TALEN-Edited MECP2 mutant cynomolgus monkeys. Cell Res. 2014;24(3):372-375.

117. Amir RE, Van den Veyver IB, Tran CQ, Francke U, Zoghbi HY. Rettsyndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet. 1999;23(2):185-188.

118. Skene PJ, Illingworth RS, Webb S, et al. Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. Mol Cell. 2010;37(4):457-468.

119. Chen Y, Yu J, Niu Y, et al. Modeling Rett syndrome using TALEN-Edited MECP2 mutant cynomolgus monkeys. Cell. 2017;169(5):945-955.

120. Newby GA, Yen JS, Woodard KJ, et al. Base editing of circulating nucleic acids. Transl Oncol. 2019;12(12):1566-1573.

121. Tseng WC, Loeb HE, Pei W, et al. Modeling Niemann-Pick disease: strategies and in vivo delivery by AAV vectors. Cell. 2020;181(1):136-150.

122. van Haasteren J, Li J, Scheideler OJ, Murthy N, Schaffer DV. The delivery challenge: fulfilling the promise of therapeutic genome editing. Nat Biotechnol. 2020;38(7):845-855.

123. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360(6387):436-439.

124. Sheng Y, Zhang L, Zhang Y, et al. A CRISPR/Cas13a-powered catalytic electrochemical biosensor for successive and highly sensitive RNA diagnostics. Biosens Bioelectron. 2021;178:113027.

125. Zhou P, Yu XF. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273.

126. Zhou W, Hu L, Ying L, Zhao Z, Chu PK, Yu XF. A CRISPR-Cas9 triggered strand displacement amplification method for ultrasensitive RNA detection. Nat Commun. 2021;12(1):5033.

127. Zhang H, Chen Y, Li Y, Chen R, Mardon G. CRISPR-engineered mosaicism rapidly reveals that loss of Rcnj13 function in mice mimics human disease phenotypes. Sci Rep. 2015;5:8366.

128. Wu Y, Liu SX, Wang F, Zeng MS. Room temperature detection of plasma Epstein-Barr virus DNA with CRISPR-Cas13. Clin Chem. 2019;65(4):591-592.

129. Fozouni P, Son S, Diaz de Leon Derby M, et al. Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. Cell. 2021;184(2):323-333.

130. van Haasteren J, Li J, Scheideler OJ, Murthy N, Schaffer DV. The delivery challenge: fulfilling the promise of therapeutic genome editing. Nat Biotechnol. 2020;38(7):845-855.
148. Sharma G, Sharma AR, Bhattacharya M, Lee S–S, Chakraborty C. CRISPR-Cas9: a preclinical and clinical perspective for the treatment of human diseases. *Mol Ther.* 2021;29(2):571-586.

149. Palaz F, Kalkan AK, Can Ö, et al. CRISPR-Cas3 system as a promising and versatile tool for cancer diagnosis, therapy, and research. *ACS Synth Biol.* 2021;10(6):1245-1267.

150. Fesnak AD, June CH, Levine BL. Engineered T cells: the promise and challenges of cancer immunotherapy. *Nat Rev Cancer.* 2016;16(9):566-581.

151. Qasim W, Zhan H, Samarasinghe S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci Transl Med.* 2017;9(374).

152. Dai H, Wu Z, Jia H, et al. Bispecific CAR-T cells targeting both CXCR4 protects CD4^+ T cells from HIV-1 infection. *Proc Natl Acad Sci U S A.* 2014;111(31):11461-11466.

153. Didigu CA, Wilen CB, Wang J, et al. Simultaneous zinc-finger nuclease editing of the HIV coreceptors CCR5 and CXCR4 protects CD4^+ T cells from HIV-1 infection. *Blood.* 2014;123(1):61-69.

154. Palmer DC, Guittard GC, Franco Z, et al. Cish actively silences tumor functions. *Cancer Immunol Immunother.* 2019;68(3):365-377.

155. Tang N, Cheng C, Zhang X, et al. TGF-β inhibition via CRISPR promotes the long-term efficacy of CAR T cells against solid tumors. *JCI Insight.* 2020;5(4).

156. Hu Z, Ding W, Zhu D, et al. TALEN-mediated targeting of HPV oncogenesameliorates HPV-related cervical malignancy. *J Clin Invest.* 2015;125(1):425-436.

157. Osborn MJ, Webber BR, Knipping F, et al. Evaluation of TCR gene editing achieved by TALENs, CRISPR/Cas9, and megaTAL nucleases. *Mol Ther.* 2016;24(3):570-581.

158. Ou L, Przybilla MJ, Ahlat O, et al. A highly efficacious ACSSynthBiol. 2021;10(6):1245-1267.

159. Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegvart DJ. Selective organ targeting (SORT) nucleases for tissue-specific mRNA delivery and CRISPR-Cas9 gene editing. *Nat Nanotechnol.* 2020;15(4):313-320.

160. Zeballos CMA, Gaj T. Next-generation CRISPR technologies and their applications in gene and cell therapy. *Trends in Biotechnol.* 2021;39(7):692-705.

161. Allers K, Hütter G, Hofmann J, et al. Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. *Blood.* 2011;117(10):2791-2799.

162. Perez EE, Wang J, Miller JC, et al. Establishment of HIV-1 resistance in CD4^+ T cells by genome editing using zinc-finger nucleases. *Nat Biotechnol.* 2008;26(7):808-816.

163. Flinn AM, Gennery AR. Adenosine deaminase deficiency: a review. *Orphanet J Rare Dis.* 2018;13(1):65.
186. Honaker Y, Hubbard N, Xiang Y, et al. Gene editing to induce FOXP3 expression in human CD4(+) T cells leads to a stable regulatory phenotype and function. Sci Transl Med. 2020;12(546):eaay6422.

187. Thompson LM. Neurodegeneration: a question of balance. Nature. 2008;452(7188):707-708.

188. Soto C, Pritzak S. Protein misfolding, aggregation, and conformational strains in neurodegenerative diseases. Nat Neurosci. 2018;21(10):1332-1340.

189. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell. 2010;140(6):918-934.

190. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer’s disease. Mol Neurodegener. 2019;14(1):32.

191. György B, Lööv C, Zaborowski MP, et al. CRISPR/Cas9 mediated disruption of the Swedish APP allele as a therapeutic approach for early-onset Alzheimer’s disease. Mol Ther Nucleic Acids. 2018;11:429-440.

192. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A. 1993;90(5):1977-1981.

193. Walker FO. Huntington’s disease. Lancet. 2007;369(9557):218-228.

194. Frank S. Treatment of Huntington’s disease. Neurotherapeutics. 2014;11(1):153-160.

195. Munoz-Sanjuan I, Bates GP. The importance of integrating basic and clinical research toward the development of new therapies for Huntington disease. J Clin Invest. 2011;121(2):476-483.

196. Cundiff PE, Anderson SA. Impact of induced pluripotent stem cells on the study of central nervous system disease. Curr Opin Genet Dev. 2011;21(3):354-361.

197. Kalia LV, Lang AE. Parkinson’s disease. Lancet. 2015;386(9996):550-556.

198. Davie CA. A review of Parkinson’s disease. Br Med Bull. 2008;86:109-127.

199. Chen Y, Dolt KS, Kriek M, et al. Engineering synucleinopathy-resistant human dopaminergic neurons by CRISPR-mediated deletion of the SNCA gene. Eur J Neurosci. 2019;49(4):510-524.

200. Qian H, Kang X, Hu J, et al. Reversing a model of Parkinson’s disease with in situ converted nigral neurons. Nature. 2020;582(7813):550-556.

201. Brown RH, Al-Chalabi A. Amyotrophic lateral sclerosis. N Engl J Med. 2017;377(2):162-172.

202. Cirulli ET, Lassaigne BN, Petrovski S, et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science. 2015;347(6229):1436-1441.

203. Mazzini L, Gelati M, Profico DC, et al. Results from Phase I clinical trial with intraspinal injection of neural stem cells in amyotrophic lateral sclerosis: a long-term outcome. Stem Cells Transl Med. 2019;8(9):887-897.

204. Teng YD, Benn SC, Kalkanis SN, et al. Multimodal actions of neural stem cells in a mouse model of ALS: a meta-analysis. Sci Transl Med. 2012;4(165):165ra164.

205. Gaj T, Ojala DS, Ekman FK, Byrne LC, Limsirichai P, Schaffer DV. In vivo genome editing improves motor function and extends survival in a mouse model of ALS. Sci Adv. 2017;3(12):eaar3952.
245. Berdeja JG, Madduri D, Usmani SZ, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTRITTIUDE-1): a phase 1b/2 open-label study. *Lancet*. 2021;398(10297):314-324.

246. Zufferey R, Dull T, Mandel RJ, et al. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol*. 1998;72(12):9873-9880.

247. Lombardo A, Genovese P, Beaunejour CM, et al. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. *Nat Biotechnol*. 2007;25(11):1298-1306.

248. Wang X, Wang Y, Wu X, et al. Unbiased detection of off-target cleavage by CRISPR-Cas9 and TALENs using integrase-defective lentiviral vectors. *Nat Biotechnol*. 2015;33(2):175-178.

249. Mátrai J, Cantore A, Bartholomae CC, et al. Hepatocyte-targeted expression by integrase-defective lentiviral vectors induces antigen-specific tolerance in mice with low genotoxic risk. *Hepatology*. 2011;53(5):1696-1707.

250. Strobel B, Zuckshwerdt K, Zimmermann G, et al. Standardized, scalable, and timely flexible adeno-associated virus vector production using frozen high-density HEK-293 cell stocks and CELLdiscs. *Hum Gene Ther Methods*. 2019;30(1):23-33.

251. Tabebordbar M, Lagerborg KA, Stanton A, et al. Directed evolution of a family of AAV capsid variants enabling potent muscle-directed gene delivery across species. *Cell*. 2021;184(19):4919-4938.

252. Fonseca-Santos B, Gremião MP, Chorilli M. Nanotechnology-based drug delivery systems for the treatment of Alzheimer's disease. *Int J Nanomedicine*. 2015;10:4981-5003.

253. Knight FC, Gilchuk P, Kumar A, et al. Mucosal Immunization with a pH-responsive nanoparticle vaccine induces protective CD8(+) lung-resident memory T cells. *ACS Nano*. 2019;13(10):10939-10960.

254. Ashfarzadeh M, Hashemi M, Mokhtarzadeh A, Abnous K, Ramezani M. Recent advances in co-delivery systems based on polymeric nanoparticle for cancer treatment. *Artif Cells Nanomed Biotechnol*. 2018;46(6):1095-1110.

255. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov*. 2018;19(7):1979.

256. Singh P, Pandit S, Mokkapati V, Garg A, Ravikumar V, et al. Recent advances in co-delivery systems based on polymeric nanoparticle for cancer treatment. *Artif Cells Nanomed Biotechnol*. 2018;46(6):1095-1110.

257. Finn JD, Smith AR, Patel MC, et al. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing and hepatitis B virus inhibition. *J Control Release*. 2021;330:61-71.

258. Ding Y, Jiang Z, Saha K, et al. Gold nanoparticles for nucleic acid delivery. *Mol Ther*. 2014;22(6):1075-1083.

259. Singh P, Pandit S, Mokkapati V, Garg A, Ravikumar V, Mijakovic I. Gold nanoparticles in diagnostics and therapeutics for human cancer. *Int J Mol Sci*. 2018;19(7):1979.

260. Chen S, Lee B, Sato R, et al. Lipid nanoparticles loaded with ribonucleoprotein-oligonucleotide complexes synthesized using a microfluidic device exhibit robust genome editing and hepatitis B virus inhibition. *J Control Release*. 2021;330:61-71.

261. Mokhtarzadeh A, Hashemi M, Mokhtarzadeh A, Abnous K, Ramezani M. Recent advances in co-delivery systems based on polymeric nanoparticle for cancer treatment. *Artif Cells Nanomed Biotechnol*. 2018;46(6):1095-1110.

262. Finn JD, Smith AR, Patel MC, et al. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. *Cell Rep*. 2018;22(9):2227-2235.

263. Ding Y, Jiang Z, Saha K, et al. Gold nanoparticles for nucleic acid delivery. *Mol Ther*. 2014;22(6):1075-1083.

264. Singh P, Pandit S, Mokkapati V, Garg A, Ravikumar V, Mijakovic I. Gold nanoparticles in diagnostics and therapeutics for human cancer. *Int J Mol Sci*. 2018;19(7):1979.
263. Schumann K, Lin S, Boyer E, et al. Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. *Proc Natl Acad Sci U S A*. 2015;112(33):10437-10442.

264. Wang J, Exline CM, DeClercq JJ, et al. Homology-driven genome editing in hematopoietic stem and progenitor cells using ZFN mRNA and AAV6 donors. *Nat Biotechnol*. 2015;33(12):1256-1263.

265. Dai X, Park JJ, Du Y, et al. One-step generation of modular CAR-T cells with AAV-Cpf1. *Nat Methods*. 2019;16(3):247-254.

266. Frangoul H, Alshuler D, Cappellini MD, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. *N Engl J Med*. 2021;384(3):252-260.

267. Stadtmauer EA, Fraietta JA, Davis MM, et al. CRISPR-engineered T cells in patients with refractory cancer. *Science*. 2020;367(6481):eaba7365.

268. Benjamin R, Graham C, Yallop D, et al. Genome-edited, donor-derived allogeneic anti-CD19 chimeric antigen receptor T cells in paediatric and adult B-cell acute lymphoblastic leukaemia: results of two phase 1 studies. *Lancet*. 2020;396(10266):1885-1894.

269. Nishiga M, Qi LS, Wu JC. Therapeutic genome editing in cardiovascular diseases. *Adv Drug Deliv Rev*. 2021;168:147-157.

270. German DM, Mitalipov S, Mishra A, Kaul S. Therapeutic genome editing in cardiovascular diseases. *JACC Basic Transl Sci*. 2019;4(1):122-131.

271. Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol*. 2013;31(9):822-826.

272. Chang HHY, Pannunzio NR, Adachi N, Lieber MR. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat Rev Mol Cell Biol*. 2017;18(8):495-506.

273. Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH. Off-target effects in CRISPR/Cas9-mediated genome engineering. *Mol Ther Nucleic Acids*. 2015;4(11):e264.

274. Vermersch E, Jouve C, Hulot JS. CRISPR/Cas9 gene-editing strategies in cardiovascular cells. *Cardiovasc Res*. 2020;116(5):894-907.

275. Kim D, Luk K, Wolfe SA, Kim JS. Evaluating and enhancing target specificity of gene-editing nucleases and deaminases. *Annu Rev Biochem*. 2019;98:191-220.

276. Zhong Z, Sretenovic S, Ren Q, et al. Improving plant genome editing with high-fidelity xCas9 and non-canonical PAM-targeting Cas9-NG. *Mol Plant*. 2019;12(7):1027-1036.

277. Miller SM, Wang T, Randolph PB, et al. Continuous evolution of SpCas9 variants compatible with non-G PAMs. *Nat Biotechnol*. 2020;38(4):471-481.

278. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. *Science*. 2016;351(6268):84-88.

279. McGrath E, Shin H, Zhang L, et al. Targeting specificity of APOBEC-based cytosine base editor in human iPSCs determined by whole genome sequencing. *Nat Commun*. 2019;10(1):5333.

280. Musunuru K, Chadwick AC, Mizoguchi T, et al. In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. *Nature*. 2021;593(7859):429-434.

281. Crudele JM, Chamberlain JS. Cas9 immunity creates challenges for CRISPR gene editing therapies. *Nat Commun*. 2018;9(1):3497.

282. Charlesworth CT, Deshpande PS, Dever DP, et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. *Nat Med*. 2019;25(2):249-254.

283. Moreno AM, Palmer N, Alemán F, et al. Immune-orthogonal orthologues of AAV capsids and of Cas9 circumvent the immune response to the administration of gene therapy. *Nat Biomed Eng*. 2019;3(10):806-816.

284. Wagner DL, Amini L, Wendering DJ, et al. High prevalence of *Streptococcus pyogenes* Cas9-reactive T cells within the adult human population. *Nat Med*. 2019;25(2):242-248.

285. Guerrini CJ, Curnutte MA, Sherkow JS, Scott CT. The rise of the ethical license. *Nat Biotechnol*. 2017;35(1):22-24.

286. Long C, McAnally JR, Shelton JM, Mireault AA, Basset-Duby R, Olson EN. Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. *Science*. 2014;345(6201):1184-1188.

287. Yue Y, Xu W, Kan Y, et al. Extensive germline genome engineering in pigs. *Nat Biomed Eng*. 2021;5(2):134-143.

288. Turcocy J, Adashi EY, Egli D. Heritable human genome editing: research progress, ethical considerations, and hurdles to clinical practice. *Cell*. 2021;184(6):1561-1574.

---

How to cite this article: Zhou W, Yang J, Zhang Y, Hu X, Wang W. Current landscape of gene-editing technology in biomedicine: Applications, advantages, challenges, and perspectives. *MedComm*. 2022;3:e155. [https://doi.org/10.1002/mco2.155](https://doi.org/10.1002/mco2.155)