Sharaf-Eldin
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REVIEW

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Technologies of gene editing and related clinical trials for the treatment of genetic and acquired diseases: a systematic review

Wessam Sharaf-Eldin[1*]

Abstract

Background Gene editing can produce irreversible permanent changes to the genetic material at predetermined sequences, avoiding random integration, which is the major drawback of classical gene therapy. The technology has invaded all approaches of genetic engineering and biotechnology with versatile applications in agriculture, industry, and medicine.

Main body The present review displays the different approaches and mechanisms of gene editing. Special emphasis has been given to the technology therapeutic applications where all registered clinical trials have been addressed. The Islamic ethical concerns of gene editing have also been highlighted.

Conclusion The great advantages of gene editing technology, coupled with the splendid efforts of scientists to develop systems with superior efficacy and safety would provide an effective avenue for treating a wide range of human diseases in the near future.

Keywords DNA DSBs, DNA repair systems, Gene editing, Peptide nucleic acids, Programmable nucleases

Background

Targeted DNA modifications are primarily accomplished by means of programmable nucleases that induce site-specific DNA double-strand breaks (DSBs). The DSBs can be restored by cellular DNA repair systems that allow the insertion, deletion, or alteration of genetic material at a particular DNA site. The programmable nucleases have further been engineered to create nickases that only cut a single DNA strand with much more precision and efficacy. Furthermore, gene editing can also be achieved without triggering any DNA breaks using synthetic nucleic acid-like molecules known as peptide nucleic acids (PNAs).

*Correspondence:
Wessam Sharaf-Eldin
wessam_sharafeldin@yahoo.com
[1] Medical Molecular Genetics Department, National Research Centre, Cairo, Egypt

The DSBs can be mainly repaired by two different mechanisms: homology-directed repair (HDR) or non-homologous end joining (NHEJ). HDR utilizes an exogenous single- or double-stranded DNA template for the repair of DSBs. Such a donor template is flanked by sequences identical to those around the break site (homology arms), incorporating their sequence variations into the cut region. This mechanism is mainly utilized for mutation repair (Fig. 1a). However, NHEJ repairs DSBs via direct religation of the cleaved ends. It is an error-prone mechanism that often induces the formation of small indels at the cut region, where it is primarily associated with specific gene knockout [1] (Fig. 1b). If two DSBs are created together on one chromosome, the intervening sequence between the two DSBs can be deleted or inverted (Fig. 1c). Chromosomal translocations can also occur if two DSBs are simultaneously generated on two different chromosomes [2] (Fig. 1d).

NHEJ is the predominant DNA repair mechanism in high eukaryotic organisms, where HDR is largely

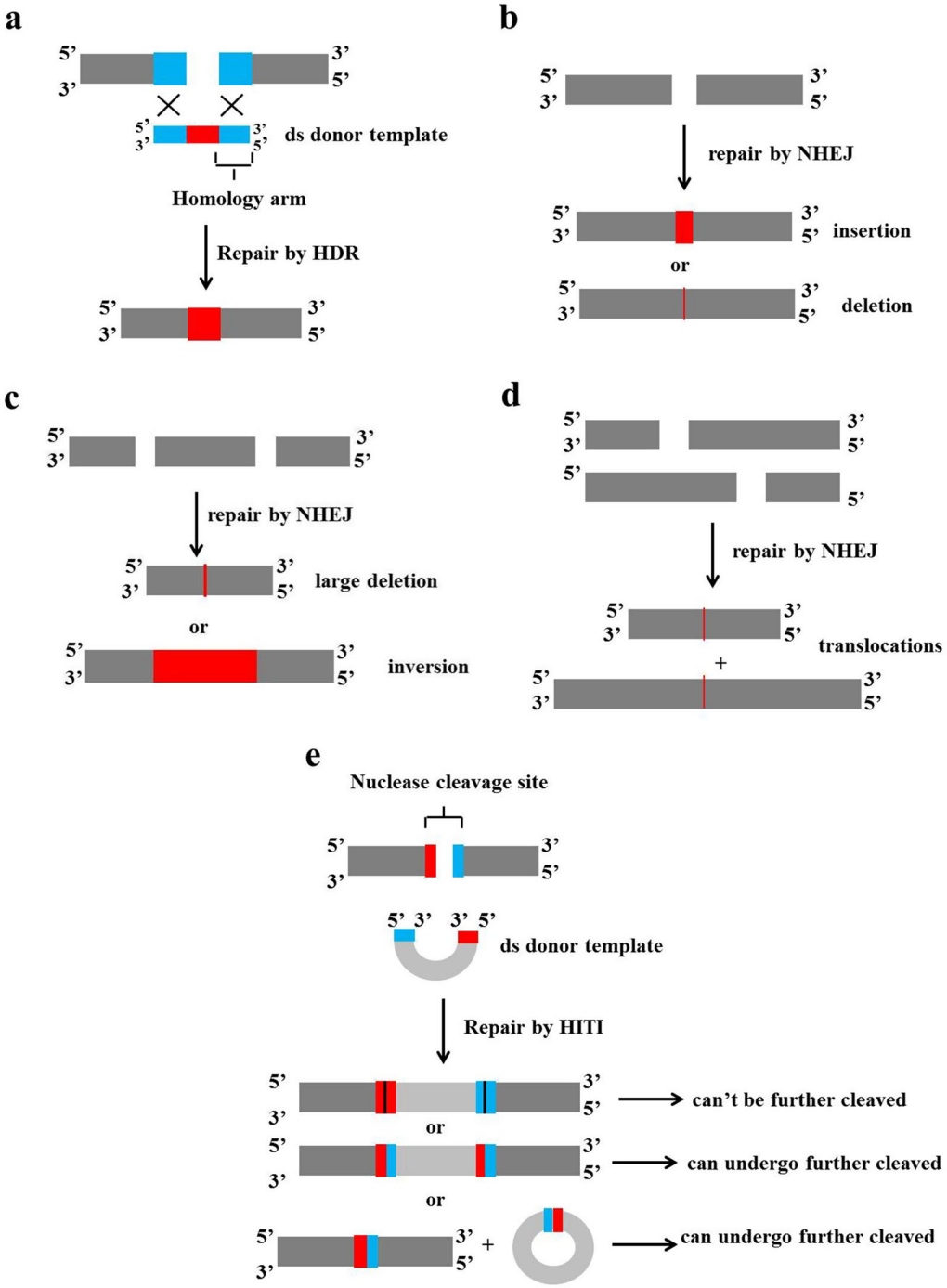

Fig. 1 Different approaches of DSB repair. **a** Repair of DSB using HDR in which donor template flanked by homology arms is provided incorporating their sequence variations into the cut region. **b** Repair of DSB by the error-prone NHEJ via direct religation of the cleaved ends forming small indels at the cut region. **c** Repair of two DSBs on the same chromosome would result in large deletion or inversion. **d** Restoring DSBs on different chromosomes might be associated with chromosomal translocations. **e** HITI can repair DSB in the presence of donor template containing the same nuclease cleavage site as the target sequence

restricted to actively dividing cells [3]. Scientists have adopted the unique strategy of homology-independent targeted insertion (HITI) for transgene insertion in both dividing and non-dividing cells. In such a situation, the target sequence as well as the donor template harbors the nuclease cleavage site, creating blunt ends. Subsequently,

the linearized donor sequence is integrated by NHEJ into the site of DSB. When incorporated in the correct orientation, the nuclease target sequence is disrupted, preventing further cleavage (Fig. 1e). Interestingly, HITI has been induced in neurons, presenting the first demonstration of gene knock-in within non-dividing cells, where it has been applied to successfully repair a certain mutation in a rat model for retinitis pigmentosa [4].

Progammable nucleases

They can be categorized into two main classes: FoKI-dependent nucleases and CRISPR-Cas systems. Each consists of a variable DNA-binding domain and a constant catalytic domain.

a. FokI-dependent nucleases

The activity of two different classes of programmable nucleases depends on the cleavage domain of the FokI restriction endonuclease: zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). ZFNs first emerged in 1991 [5], representing the master gene editing tool for about 20 years until TALENs were engineered in 2011 [6]. Each class contains a customized array of different DNA-binding domains: zinc finger proteins (ZFPs) and transcription activator-like effectors (TALEs) in ZFNs and TALENs, respectively. FokI must dimerize to cleave DNA; therefore, two nuclease monomers, separated by a short spacer, bind the complementary DNA strands (Figs. 2a and 1b).

In an attempt to enhance the nuclease efficiency, FokI nickases, in which the catalytic activity of one FokI monomer is inactivated, have been developed. Accordingly, nickases produce single-strand breaks (SSBs) or nicks instead of DSBs, which are mainly repaired by HDR rather than NHEJ, which authorize highly precise gene editing [7] (Figs. 2c and 1d). Custom design of FokI-dependent nucleases requires substantial protein engineering, which is laborious, time-consuming, and expensive. Besides, they can introduce DSBs at untargeted genomic sites (off-target sites), leading to non-specific DNA modifications including point mutations, deletions, insertions, inversions, and translocations.

b. CRISPR-Cas systems

CRISPR-Cas (Clustered regularly interspaced short palindromic repeats and CRISPR-associated enzyme) systems have been classified into two major classes (class I and class 2), each of which includes several types and subtypes (Table 1). Class 2 systems use only one Cas protein, which is most commonly applied in gene editing. Noteworthy, type VI Cas13 targets RNA instead of DNA, providing new avenues for gene editing. On the other hand, class 1 systems utilize a complex of multiple Cas proteins [8]. They have also recently emerged as possible tools for gene editing technologies with potential characteristics [9].

CRISPR-Cas9 of *Streptococcus pyogenes* (SpCas9) was the first type II system to be discovered, characterized, and utilized in gene editing [10]. CRISPR regions consist of a CRISPR RNA (crRNA) containing 20-bp spacer that binds the DNA target sequence and a trans-activating CRISPR RNA (tracrRNA) that interacts with the crRNA to generate functional small guide RNA (sgRNA). Once sgRNA is formed, it can be complexed with Cas9, enabling the nuclease to bind DNA via the crRNA. Cas9 also binds DNA via a 3-bp protospacer adjacent motif (PAM), predominantly 5'-NGG-3'. Cas9 contains two catalytic domains, RuvC and HNH; each nicks a single strand of the target DNA. The HNH domain cuts the sgRNA complementary strand, and the RuvC domain cleaves the displaced one generating, a site-specific DSB [11] (Fig. 2e). Importantly, inactivation of one of the Cas9 catalytic domains results in a partially inactivated Cas9 or Cas9 nickase that can only generate SSB (Figs. 2f and g), whereas inactivation of both domains inhibits the nuclease catalytic activity, resulting in dead Cas9 (dCas9) [12] (Fig. 2h).

The applications of dCas9 extend beyond gene editing by virtue of its guidable capacity. It can recruit various effector proteins to specific DNA sequences, mediating several manipulations. These guidable proteins include transcriptional activators and repressors (to regulate gene expression), epigenetic modulators (to trigger epigenetic changes), chromatin rewiring proteins (to modify the 3D chromatin structure), and fluorescently labeled proteins (for chromatin imaging) [13].

CRISPR-Cas systems have surpassed other programmable nucleases due to their easy design, low cost, and higher efficiency. Since emerging, CRISPR-based gene editing has progressed at an unprecedented pace, being utilized in the majority of gene editing systems in the past few years. By 2020, the CRISPR/Cas systems have been utilized in all announced clinical trials. In 2015, CRISPR-Cas9 was named the "Breakthrough of the Year" by Science magazine. In 2020, the Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for Crispr gene editing [14].

The major concern with CRISPR-Cas systems is their off-target effects. In fact, CRISPR-Cas tools have higher off-target activity than other nucleases. Several strategies have been developed to mitigate the CRISPR/Cas9 off-target effect, including sgRNA optimization, Cas9 modification, application of other Cas variants, and inhibition of Cas enzymes. Several academic and commercial online resources with user-friendly interfaces are currently available to select and design optimal sgRNAs, where

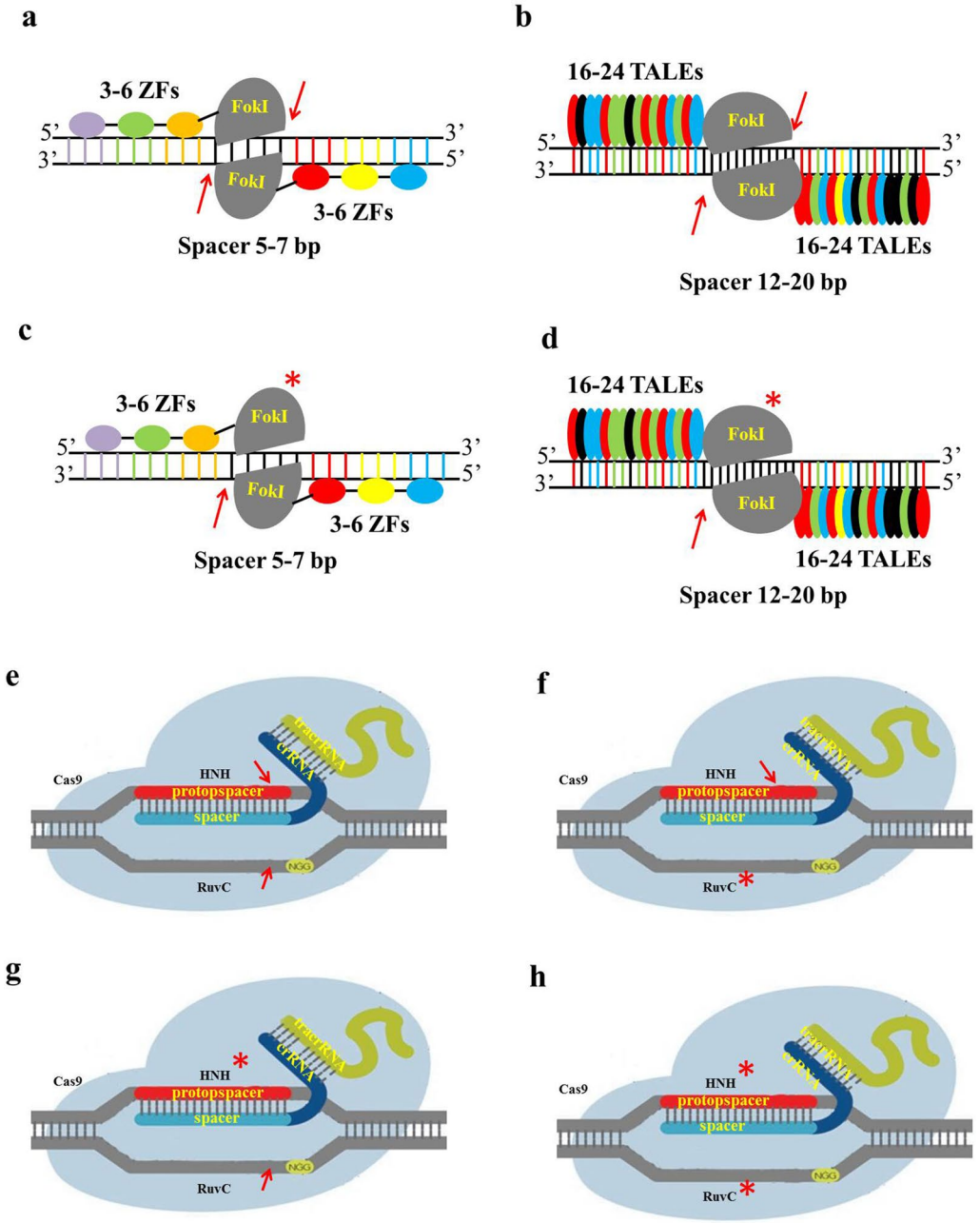

Fig. 2 Programmable nucleases. **a** ZFN: the monomer contain 3–6 zinc fingers (ZFs); each interacts with 3 DNA bases binding DNA sequences of 9–18 bp, where the active dimer achieves a specificity of 18–36 bp. ZFs recognizing 47 of all possible 64 DNA triplets have been characterized. **b** TALEN: each TALE consists of 33–39 amino acids binding a single base pair. The nucleotide specificity of each TALE is determined by the two amino acids at positions 12 and 13, known as repeat variable diresidues (RVDs). Four different RVDs specifically, Asn–Ile, His–Asp, Asn-His, and Asn–Gly, are most widely applied to recognize adenine, cytosine, guanine and thymine residues, respectively. TALEN target site must have a 5′T residue, specified by the constant N-terminal TALE. The TALEN monomer binds DNA sequences of 16–24 bp (32–48 bp when upon dimerization). **c** ZFN nickase: in which the catalytic activity of one FokI monomer is inactivated. **d** TALEN nickase. **e** Wild type Cas9 cleaves both DNA strands. **f** Cas9 D10A nickase with inactivated RuvC domain cleaving the target strand only. **g** Cas9 H840A nickase with inactivated HNH domain cleaving the non-target strand only. **h** dCas9 without any nuclease activity. Red stars denote for inactive catalytic domains and red small arrows indicate the cut sites

Table 1 CRISPR-Cas systems types and subtypes

Type	Class I			Class II		
	I	III	IV	II	V	VI
Nuclease	Cas3	Cas10	DinG	Cas9	Cas12, Cas14	Cas13
Target	DNA	DNA, RNA	Unknown	DNA	DNA	RNA

GC content and length should be considered. Moreover, chemical modifications of sgRNAs, including base, sugar, and backbone modifications, can enhance system efficiency and reduce off-target activity [15]. Modification of the Cas-9 protein involves the use of Cas9 nickases and high-fidelity Cas9 variants. The high-fidelity Cas9 variants are generated by introducing certain amino acid substitutions in the Cas9 groove lying between its nuclease domains. This groove mediates the Cas binding with the PAM-containing DNA strand. As this groove is positively charged, it has a strong electrostatic binding with the negatively charged DNA, irrespective of the sequence homology. Therefore, decreasing the groove electropositivity would contribute to reduced off-target activity. These variants include eSpCas9, SpCas9-HF1, HypaCas9, Sniper-Cas9, xCas9, and evoCas9 [16]. Given their superior specificity, it is likely that these variants will be adopted as the future gold-standard tools for gene editing. On the other hand, different Cas9 orthologs derived from other bacterial species as well as other Cas proteins, such as Cas-12a, Cas-3, and Cas-10, have provided better gene editing efficiency than SpCas-9 [17]. Inhibition of Cas enzymes can be mediated by anti-CRISPR (Acr) proteins, small-molecule Cas inhibitors, and small nucleic acid-based CRISPR inhibitors, preventing sustained nuclease activity and reducing the off-target effects [8].

DSB-independent gene editing strategies

a. Base editing

Base editing is a unique approach to gene editing that enables targeted substitution of a specific DNA base. The first system of base editors was developed by Komor et al. in 2016 [18]. Base editors (BEs) are composed of cytidine or adenosine deaminase enzyme fused to a CRISPR/Cas system, forming cytosine base editors (CBEs) or adenine base editors (ABEs), respectively. BEs normally mediate nucleotide transitions, i.e., purine to purine or pyrimidine to pyrimidine, where cytidine and adenosine are converted into uridine and inosine finally replaced by thymidine and guanosine, respectively (Fig. 3a). Inosine is automatically recognized as guanosine by the cellular machinery, while uridine, which is not a natural DNA nucleoside, will be removed from DNA by uracil DNA glycosylase (UDG). Therefore, uracil DNA glycosylase inhibitor (UGI) has been incorporated with CBEs, forming glycosylase base editors (GBEs).

The first CRISPR/Cas system used in base editors was dCas9. However, Cas9 nickase has subsequently demonstrated more efficient gene editing, where the edited strand can be used as a template to repair the nicked strand and/or provide better deaminase accessibility [19]. Targeting cytosine and adenine bases occurs within an activity window of about 5 nucleotides on the non-target strand [20]. Interestingly, both CBEs and ABEs have been recently modified to induce specific DNA transversions [21, 22]. However, base editing is unable to introduce small insertions or deletions.

b. Prime editing

The technology of prime editing has been recently described by Anzalone and his colleagues in 2019 [23]. Prime-editors (PEs) consist of Cas9 nickase fused to engineered reverse transcriptase (RT) in addition to unique prime-editing guide RNA (pegRNA). The pegRNA contains sgRNA, a primer binding site, and an RT template with edit. The Cas9 nickase cuts the PAM containing DNA strand, liberating a free 3'-hydroxyl group that induces the reverse transcription of the pegRNA RT template. This yields a branched intermediate containing two DNA flaps: the edited 3' flap and the unedited 5' flap. The cellular DNA repair system can integrate any of these two flaps into the genomic DNA. Interestingly, PEs can be engineered to modify the PAM sequence to prevent further editing when the modified 3' flap is incorporated into the host genome. However, if the original sequence is restored again to the target site, a new cycle of prime editing can be initiated.

c. Peptide nucleic acids (PNAs)

PNAs, first generated in 1991, are synthetic nucleic acid analogues in which the sugar phosphate backbone of nucleic acids is replaced by a synthetic polyamide (protein-like) backbone of N-(2-amino-ethyl)-glycine [24]. Such modification has granted PNAs high stability and resistance against cleavage by proteases and nucleases. PNAs can form duplexes with DNA and RNA target sequences obeying the Watson–Crick base pairing model. They are highly specific, where few mismatches are associated with inefficient target binding. The PNA

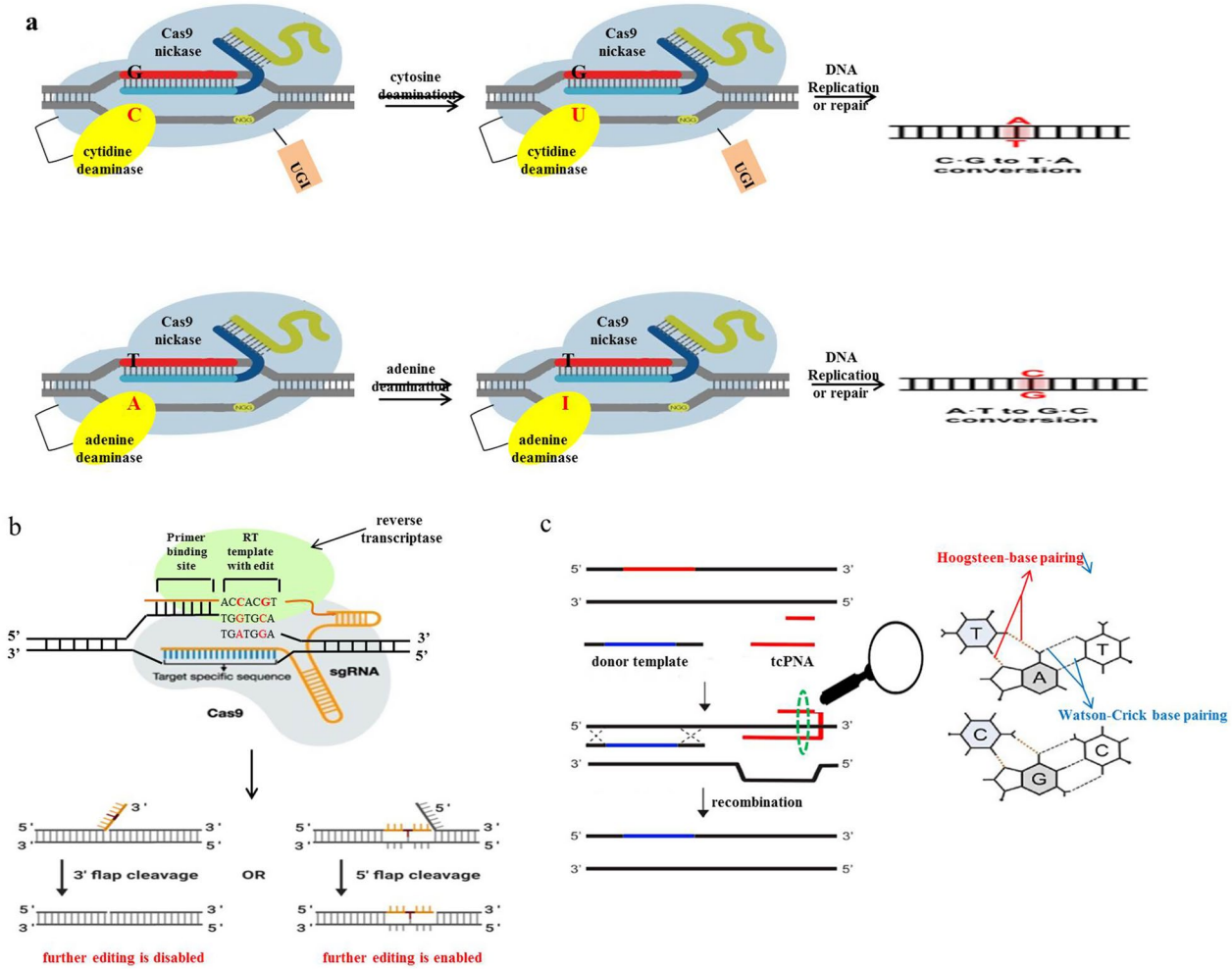

Fig. 3 DSB-independent gene editing strategies. **a** Base editing: Cytosine base editors (CBEs) convert cytidine into uridine finally replaced by thymidine, thereby the base pairs of C–G are converted into T–A, while adenine base editors (ABEs) convert adenosine into inosine finally replaced by guanosine. Accordingly, ABEs enable the transition of A–T into G–C. UGI: uracil DNA glycosylase inhibitor. **b** Prime editing: Prime-editors (PEs) consist of Cas9 nickase, reverse transcriptase (RT) and prime-editing guide RNA (pegRNA). The pegRNA contains sgRNA, primer binding site and RT template with edit. The Cas9 nickase cuts the DNA strand activating the reverse transcription of the pegRNA RT template forming an intermediate with two flaps. The DNA repair system can integrate either the edited 3′ flap or the unedited 5′ flap. Only upon incorporation of the 3′ flap, further editing is prohibited. **c** PNA-mediated gene editing: Tail-clamp PNA (tcPNA) interacts with a single DNA strand, where one PNA strand associates with a polypurine DNA stretch by Hoogsteen-base pairing and the second strand binds DNA by Watson–Crick base pairing. PNA-DNA binding displace the two DNA strands apart enabling recombination with ssDNA donor template at a nearby location

neutral charge strengthens the PNA/DNA complexes compared to those of DNA/DNA. This effect is extensively enhanced when two PNAs interact with a single DNA strand. In such a situation, one PNA strand associates with a polypurine DNA stretch along the helix major groove by Hoogsteen-base pairing, and the second strand binds DNA by Watson–Crick base pairing. Subsequently, PNAs open the DNA helical structure, stimulating site-specific recombination at a nearby genomic location using an exogenous ssDNA donor template. As PNAs lack nuclease activity, their safety profile is critically enhanced, where their off-target activity is very low or even undetected [25].

The first PNA applied in gene editing was bis-PNA, in which two PNA molecules with the same length were connected by a linker sequence [26]. Subsequently, different types of PNAs have been designed. However, a tail-clamp PNA (tcPNA), in which the Watson–Crick part is extended over the Hoogsteen part, and its chemically modified derivative, γtcPNA, have been found to be associated with superior activity [27]. Interestingly, PNA can also mediate single-base modification without the use

of donor DNA. In such cases, ssPNAs, which are different from the target sequence for only a single base, are employed. Thereby, PNA is not only acting by stimulating recombination with donor DNA but also by acting as a source of genetic information [28]. PNAs have some advantages over programmable nucleases due to their small size, powerful specificity, and extreme safety. However, they are associated with relatively low efficiency. Their application to correct the cystic fibrosis gene in mice has achieved 9% efficiency when inhaled and only 1% when injected [29]. PNA might be suitable for therapeutic application when the correction of a small cell subset would be significant. For example, editing only 6–7% of cells was found to potentially ameliorate mice with beta thalassemia [30]. On the other hand, advances in PNA structure and delivery systems would contribute to more efficient applications.

Delivery systems

Programmable nucleases can be delivered in the form of DNA plasmids, in-vitro transcribed mRNAs (Cas mRNA and sgRNA in the case of CRISPR/Cas systems), or purified proteins (ribonucleoproteins; RNPs, in the case of CRISPR/Cas systems). DNA-based delivery allows long-lasting nuclease expression; however, it is associated with increased off-target activity. However, when nucleases are delivered as mRNA or proteins, they induce on-target mutations shortly after delivery and degrade rapidly, reducing the off-target effects [31].

There are three main classes of gene editing delivery systems: physical, chemical, and viral platforms. Generally, non-viral (physical and chemical) methods have lower efficiency than viral vectors. The physical methods include electroporation, microinjection, and hydrodynamic injection. Electroporation applies an intense electric field to the cell membrane to increase its permeability for a while enabling the influx of the gene editing tool into cells. Microinjection is a direct microscopic injection of the effector into the target cells. Hydrodynamic injection involves the immediate injection of a huge volume of high-pressure liquid into the animal blood stream via the tail vein, mediating delivery to liver cells specifically. The chemical vectors mainly comprise lipid and polymer nanoparticles. Both allow cell entry of the gene editing tool via endocytosis.

Viral vectors include lentiviral vectors (LVs), adenoviral vectors (AVs), and adeno-associated viral vectors (AAVs). AAVs are characterized by their reduced immunogenicity and non-integration into the host genome, representing the most efficient delivery system. However, its cloning capacity is limited to < 4.8 kb, impeding their application for delivery of large nucleases, such as TALENs and *Sp*Cas9. Several strategies have been adopted to tackle this hurdle, including: 1) packaging *Sp*Cas9 and sgRNA into separate AAVs and transfecting them into cells simultaneously; 2) using truncated *Sp*Cas9; and 3) using smaller Cas9 orthologs such as *Staphylococcus aureus* Cas9 (*Sa*Cas9).

Therapeutic applications of gene editing

Gene editing technology can be utilized to irreversibly restore or disrupt the function of specific target genes for therapeutic purposes. Gene editing of autologous T cells, hematopoietic stem cells (HSCs), and induced pluripotent stem cells (iPSCs) would provide an efficient strategy of personalized transplantation for treating various diseases and disorders. Generally, the clinical application of gene technology involves either ex vivo platform in which the patient's cells are isolated, edited in vitro, and then delivered back to the human body or in vivo platform in which the gene editing system is directly delivered to the patient's target cells or tissue.

Therapeutic platforms of gene editing are exceedingly applied in almost all types of human diseases, including viral infections [32], cancer [33], blood disorders [34], primary immunodeficiencies [35], inborn errors of metabolism [36], muscular disorders [37–39], congenital lung diseases [40], neurological disorders [41–44], dermatological disorders [45], inherited eye diseases [46] and hereditary hearing loss [47]. To date, more than 80 clinical trials have already been registered on ClinicalTrials.gov. They are mainly designed to target a group of monogenic disorders, type 1 diabetes mellitus (T1D), certain viral infections, and different malignant transformations (Table 2).

a. Monogenic disorders

Clinical trials of monogenic disorders are primarily related to sickle cell disease (SCD) and β-thalassemia caused by abnormal or insufficient hemoglobin (Hb) production due to mutations in the hemoglobin beta gene (HBB). To date, there are 18 ex vivo clinical trials on SCD ($n=7$) and β-thalassemia ($n=11$), of which six have progressed to phase 3. They depend on either the correction of HBB mutations or reactivating the HBG genes, increasing the production of fetal hemoglobin (HbF: α2γ2)and compensating for adult hemoglobin (HbA: α2β2). Reactivating the HBG gene can be accomplished by removing the cis-regulatory elements of the HBG1/2 promoter region or inactivating the expression of the HbF production inhibitory gene (BCL11a) [48].

Ten trials have also been conducted on other genetic disorders (one for each). All of them involve the in vivo application of the gene editing complex to the target cells. Interestingly, five studies have launched in 2022 and 2023, and it is expected that the number of trials

Table 2 Clinical trials based on gene editing technology

Series	Identifier-location	Condition	Target cells	Intervention—drug	Editor—approach	Start date -status	Phase
Monogenic disorders							
1	NCT04819841—US	SCD	Autologous HSCs	*HBB* correction—GPH101	CRISPR/Cas9—ex vivo	15/11/2021—terminated	1/2
2	NCT04774536—US	SCD	Autologous HSCs	*HBB* correction—CRISPR_ SCD001	CRISPR/Cas9—ex vivo	1/6/2024—not yet recruiting	1/2
3	NCT04853576—US	SCD	Autologous HSCs	HBG1/2 promoter editing—EDIT-301	CRISPR/Cas9—ex vivo	4/5/2021—recruiting	1/2
4	NCT03653247—US	SCD	Autologous HSPCs	BCL11A disruption—BIVV003	ZFN—ex vivo	6/3/2019—recruiting	1/2
5	NCT03745287—US	SCD	Autologous HSPCs	BCL11A disruption—CTX001	CRISPR/Cas9—ex vivo	27/11/2018—active not recruiting	2/3
6	NCT05329649—US	SCD	Autologous HSPCs	BCL11A disruption—CTX001	CRISPR/Cas9—ex vivo	2/5/2022—recruiting	3
7	NCT05951205—not provided	SCD	Autologous HSPCs	BCL11A disruption—CTX001	CRISPR/Cas9—ex vivo	1/2024—not yet recruiting	3
8	NCT03728322 – not provided	TDT	Autologous iHSCs	*HBB* correction	CRISPR/Cas9—ex vivo	1/2019—unknown	1
9	NCT04205435—China	TDT	Autologous HSCs	*HBB* correction	CRISPR/Cas9—ex vivo	1/11/2021—terminated	1/2
10	NCT05444894—US	TDT	Autologous HSCs	HBG1/2 promoter editing – EDIT-301	CRISPR/Cas9—ex vivo	29/4/2022—recruiting	1/2
11	NCT05442346—China	TDT	Autologous HSCs	HBG1/2 promoter editing	glycosylase base editors – ex vivo	25/12/2023—suspended	NA
12	NCT03432364—US	TDT	Autologous HSPCs	BCL11A disruption—ST-400	ZFN—ex vivo	29/3/2018—completed	1/2
13	NCT03655678—US	TDT	Autologous HSPCs	BCL11A disruption—CTX001	CRISPR/Cas9—ex vivo	14/9/2018—active not recruiting	2/3
14	NCT05356195 – US, Germany, Canada & Italy	TDT	Autologous HSPCs	BCL11A disruption—CTX001	CRISPR/Cas9 – ex vivo	3/5/2022—recruiting	3
15	NCT05477563—US, Germany,	TDT	Autologous HSPCs	BCL11A disruption—CTX001	CRISPR/Cas9—ex vivo	2/8/2022—recruiting	3
16	NCT04925206—China	TDT	Autologous HSPCs	BCL11A disruption—ET-01	CRISPR/Cas9—ex vivo	17/8/2021- active not recruiting	1
17	NCT04211480—China	TDT	Autologous HSPCs	BCL11A disruption	CRISPR/Cas9—ex vivo	1/4/2020—active not recruiting	NA
18	NCT05577312—China	TDT	Autologous HSPCs	BCL11A disruption—BRL-101	CRISPR/Cas9—ex vivo	1/11/2022—recruiting	1
19	NCT02695160—US	hemophilia B	Hepatocytes—albumin locus	Insertion of correct copy of gene encoding factor IX—SB-FIX	ZFN – in vivo	15/11/2016—terminated	1
20	NCT02702115—US	MPSI	Hepatocytes—albumin locus	Insertion of correct copy of *IDUA*—SB-318	ZFN – in vivo	24/5/2017—terminated with results	1/2

Table 2 (continued)

Series	Identifier-location	Condition	Target cells	Intervention—drug	Editor—approach	Start date -status	Phase
21	NCT03041324—US	MPSII	Hepatocytes—albumin locus	Insertion of correct copy of *I2S* – SB-913	ZFN – in vivo	11/5/2017—terminated with results	1/2
22	NCT05222178—US	PKU	Liver cells	Insertion of correct copy of *PAH* – HMI-103	not provided – in vivo	3/6/2022 – active not recruiting	1
23	NCT05514249—US	DMD	Skeletal and cardiac muscles	treat mutations affecting the muscle promoter and first exon of dystrophin—CRD-TMH-001	CRISPR/Cas 9 – in vivo	31/8/2022—active not recruiting	1
24	NCT06025032—China	*OTOF* related CHL	Inner hair cells	Repair of p.Q829X mutation in *OTOF*—HG205	CRISPR/Cas13 RNA base editing – in vivo	30/11/2023—not yet recruiting	1
25	NCT03872479—US	LCA10	Photoreceptor cells	Repair of c.2991 + 1655 A > G mutation in *CEP290*—EDIT-101	CRISPR/Cas9 – in vivo	26/9/2019—active not recruiting	1/2
26	NCT05805007—not provided	*RHO* related RP	Photoreceptor cells	*RHO* silencing – ZVS203e	CRISPR/Cas13 – in vivo	6/2023—not yet recruiting	1
27	NCT05398029—New Zealand & UK	HeFH	Liver cells	*PCSK9* knockout—VERVE-101	CRISPR/Cas9 – in vivo	5/7/2022—recruiting	1
28	NCT05120830—France & Germany	HAE-3	Liver cells	KLKB1 disruption—NTLA-2002	CRISPR/Cas 9 – in vivo	10/12/2021—recruiting	1/2
Diabetes mellitus type 1							
29	NCT05210530—Canada	T1D	Allogeneic pancreatic endoderm cells	Editing of immune-evasive genes – VCTX210A	CRISPR/Cas9—ex vivo	24/1/2022—completed	1
30	NCT05565248—Canada	T1D	Allogeneic pancreatic endoderm cells	editing of immune-evasive genes – VCTX211	CRISPR/Cas9—ex vivo	20/1/2023—recruiting	1/2
Viral infections							
31	NCT00842634—US	HIV	Autologous CD4 + T cells	CCR5 disruption – SB-728	ZFN—ex vivo	1/2009—completed	1
32	NCT01044654—US	HIV	Autologous CD4 + T cells	CCR5 disruption – SB-728-T	ZFN—ex vivo	12/2009—completed	1
33	NCT01252641—US	HIV	Autologous CD4 + T cells	CCR5 disruption – SB-728-T	ZFN—ex vivo	11/2010—completed	1/2
34	NCT01543152—US	HIV	Autologous CD4 + T cells	CCR5 disruption – SB-728-T	ZFN—ex vivo	12/2011—completed with results	1/2
35	NCT02225665—US	HIV	Autologous CD4 + T cells	CCR5 disruption – SB-728mR-T	ZFN—ex vivo	8/2014—completed with results	1/2
36	NCT02388594—US	HIV	Autologous CD4 + T cells	CCR5 disruption – SB-728mR	ZFN—ex vivo	4/2015—completed	1
37	NCT02500849—US	HIV	Autologous CD34 + HSPCs	CCR5 disruption – SB-728mR	ZFN—ex vivo	10/3/2016—active not recruiting	1
38	NCT03164135—China	HIV	Autologous CD34 + HSPCs	CCR5 disruption – ex vivo	CRISPR/Cas9—ex vivo	30/5/2017—unknown	NA
39	NCT03666871—US	HIV	Autologous CD4 + T cells	CCR5 disruption – SB-728-T	ZFN—ex vivo	12/6/2019 – active not recruiting	1/2

Table 2 (continued)

Series	Identifier-location	Condition	Target cells	Intervention—drug	Editor—approach	Start date -status	Phase
40	NCT03617198—US	HIV	Autologous CD4-specific CAR-T cells	CCR5 disruption – SB-728mR	ZFN—ex vivo	31/7/2019—active not recruiting	1
41	NCT05144386—US	HIV	Aviremic HIV-1 infected adults	HIV genome editing – EBT-101	CRISPR/Cas9 – in vivo	24/1/2022—recruiting	1
42	NCT04990557 – not provided	COVID-19	Virus-reactive CD8 + memory T cells	PD-1 & ACE2 knockout	CRISPR/Cas9—ex vivo	8/2021—unknown	1/2
43	NCT04560790—China	Refractory herpetic viral keratitis	Corneal cells	UL8 & UL29 destruction – BD111	CRISPR/Cas9—in vivo	4/11/2020—completed	NA
44	NCT03057912—China	HPV-related malignant neoplasm	Cervical intraepithelial neoplasia	E6 and E7 disruption	TALEN & CRISPR/Cas9—in vivo	15/1/2018—unknown	1
45	NCT02800369—China	HPV-related malignant neoplasm	Cervical intraepithelial neoplasia	E7 disruption	ZFN—in vivo	10/12/2016—unknown	1
46	NCT03226470—China	HPV-related malignant neoplasm	Cervical precancerous lesions	E6 and E7 disruption	TALEN—in vivo	10/3/2017—recruiting	1
Cancer							
47	NCT03044743—China	EBV + advanced stage malignancies	EBV-CTL	PD-1 knockout	CRISPR/Cas9—ex vivo	7/4/2017—unknown	1/2
48	NCT02793856—China	Metastatic non-small cell lung cancer	Autologous T cells	PD-1 knockout	CRISPR/Cas9—ex vivo	26/8/2016 –completed with results	1
49	NCT04417764—China	Advanced hepatocellular carcinoma	Autologous T cells	PD-1 knockout	CRISPR/Cas9—ex vivo	20/6/2019—recruiting	1
50	NCT02863913—China	Muscle-invasive bladder cancer	Autologous T cells	PD-1 knockout	CRISPR/Cas9—ex vivo	9/2016—withdrawn	1
51	NCT02867345—China	Castration-resistant prostate cancer	Autologous T cells	PD-1 knockout	CRISPR/Cas9—ex vivo	11/2016—withdrawn	1
52	NCT02867332—not provided	Metastatic renal cell carcinoma	Autologous T cells	PD-1 knockout	CRISPR/Cas9—ex vivo	11/2016—withdrawn	1
53	NCT03081715—China	Advanced esophageal cancer	Autologous T cells	PD-1 knockout	CRISPR/Cas9—ex vivo	14/3/2017—completed	1
54	NCT04426669—US	Metastatic gastro-intestinal cancer	Tumor infiltrating lymphocytes	CISH knockout	CRISPR/Cas9—ex vivo	15/5/2022—recruiting	1/2
55	NCT05566223—US	Metastatic non-small cell lung cancer	Tumor infiltrating lymphocytes	CISH knockout	CRISPR/Cas9—ex vivo	2/2023—not yet recruiting	1/2
56	NCT03399448—US	Melanoma, multiple myeloma, myxoid/round cell liposarcoma, synovial sarcoma	NY-ESO-1-specific T cells	TRAC is knocked out by introducing anti-NY-ESO-1. TRBC & PD-1 were also disrupted	CRISPR/Cas9—ex vivo	5/9/2018—terminated	1

Table 2 (continued)

Series	Identifier-location	Condition	Target cells	Intervention—drug	Editor—approach	Start date -status	Phase
57	NCT05066165—US	Acute myeloid leukemia	WT1-specific T cells	TRAC is knocked out by introducing anti-WT1. TRBC was also disrupted—NTLA-5001	CRISPR/Cas9—ex vivo	17/12/2021—terminated	1/2
58	NCT04037566—China	CD19 + leukemia or lymphoma	CD19-specific CAR-T cells	anti-CD-19 introduction & HPK1 knockout	CRISPR/Cas9—ex vivo	8/2019—recruiting	1
59	NCT03747965—China	Mesothelin + multiple solid tumors	mesothelin-directed CAR-T Cells	anti-mesothelin insertion & PD-1 knockout	CRISPR/Cas9—ex vivo	11/2018—unknown	1
60	NCT04976218—China	EGFR + solid tumors	EGFR-specific CAR-T Cells	anti-EGFR introduction & TGFβR2 knockout	CRISPR/Cas9—ex vivo	15/3/2022- recruiting	1
61	NCT04213469—China	Relapsed/refractory B cell lymphoma	CD19-specific CAR-T cells	Anti-CD-19 introduction & PD1 knockout	CRISPR/Cas9—ex vivo	13/3/2022—active not recruiting	NA
62	NCT04767308—not provided	Relapsed/refractory CD5 + hematopoietic malignancies	CD5-specific CAR-T cells	Anti-CD5 introduction & CD5 knockout	CRISPR/Cas9—ex vivo	3/2021—not yet recruiting	1
63	NCT03190278—US	Relapsed/refractory acute myeloid leukemia	CD123-specific CAR-T cells	Anti-CD123 insertion & TRAC knockout—UCART123	TALEN—ex vivo	19/6/2017—recruiting	1
64	NCT04106076—not provided	Adverse genetic risk acute myeloid leukemia	CD123-specific CAR-T cells	anti-CD123 insertion & TRAC knockout—UCART123	TALEN—ex vivo	11/7/2019—withdrawn	1
65	NCT05631912—China	Relapsed/refractory B cell non-hodgkin lymphoma	CD19-targeting CAR-T cells	Anti-CD19 insertion & TRAC knockout	CRISPR/Cas9—ex vivo	30/6/2023- recruiting	1/2
66	NCT04502446—US	Relapsed/refractory T or B cell malignancies	CD70-specific CAR-T cells	Anti-CD70 insertion & both TRAC & B2M Knockout—CTX130	CRISPR/Cas9—ex vivo	31/7/2020—recruiting	1
67	NCT04438083—US	Relapsed/refractory renal cell carcinoma	CD70-specific CAR-T cells	Anti-CD70 insertion & both TRAC & B2M Knockout—CTX130	CRISPR/Cas9—ex vivo	16/6/2020—active not recruiting	1
68	NCT05795595—US	Relapsed/refractory solid tumors	CD70-specific CAR-T cells	Anti-CD70 insertion & both TRAC & B2M Knockout—CTX131	CRISPR/Cas9—ex vivo	13/3/2023- recruiting	1/2
69	NCT04035434—US	Relapsed/refractory B cell malignancies	CD19-specific CAR-T cells	Anti-CD19 insertion & both TRAC & B2M knockout—CTX110	CRISPR/Cas9—ex vivo	22/7/2019—recruiting	1/2
70	NCT05643742—US	Relapsed/refractory B cell malignancies	CD19-specific CAR-T cells	Anti-CD19 insertion & both TRAC & B2M knockout—ex vivo—CTX112	CRISPR/Cas9—ex vivo	10/3/2023- recruiting	1/2
71	NCT04244656—US	Relapsed/refractory multiple myeloma	TNFRSF17-specific CAR-T cells	Anti-TNFRSF17 insertion & both TRAC & B2M knockout—CTX120	CRISPR/Cas9—ex vivo	22/1/2022—active not recruiting	1

Table 2 (continued)

Series	Identifier-location	Condition	Target cells	Intervention—drug	Editor—approach	Start date -status	Phase
72	NCT04637763—US	Relapsed/refractory B cell non-hodgkin lymphoma	CD19-specific CAR-T cells	Anti-CD19 insertion & both TRAC & PD-1 knock-out—CB-010	CRISPR/Cas9—ex vivo	25/6/2021—recruiting	1
73	NCT04557436—UK	CD19 + B cell leukaemia	CD19-specific CAR-T cells	Anti-CD19 insertion & both TRAC & CD52 knockout—TT52CAR19	CRISPR/Cas9—ex vivo	12/8/2020—active not recruiting	1
74	NCT04142619—US	Relapsed/refractory multiple myeloma	CD19–specific CAR-T cells	Anti-CD19 insertion & both TRAC & CS1 knock-out—UCART	TALEN—ex vivo	21/11/2019—recruiting	1
75	NCT04150497—US	Relapsed/refractory CD22 + B-cell acute lympho-blastic leukemia	CD22–specific CAR-T cells	Anti-CD22 insertion & both TRAC & CD52 knock-out—UCART22	TALEN—ex vivo	14/10/2019—recruiting	1
76	NCT03166878—China	Relapsed/refractory CD19 + leukemia and lym-phoma	CD19-specific CAR-T cells	Anti-CD19 insertion & both TRAC & B2M knock-out—UCART019	CRISPR/Cas9—ex vivo	6/2017—unknown	1/2
77	NCT03545815—China	Mesothelin + multiple solid tumors	Mesothelin-directed CAR-T Cells	Anti-mesothelin insertion & both TRAC & PD-1 knockout	CRISPR/Cas9—ex vivo	19/3/2018—unknown	1
78	NCT05722418—US	Relapsed/refractory multiple myeloma	BCMA-specific CAR-T cells	Anti-BCMA insertion & both TRAC & B2M knock-out—CB-011	CRISPR/Cas9—ex vivo	6/2/2023—recruiting	1
79	NCT06014073—China	Relapsed/refractory B cell non-hodgkin lymphoma	CD19-directed CAR-T cells	Anti-CD19 insertion & both TRAC & Power3 knockout	CRISPR/Cas9—ex vivo	1/9/2023 not yet recruiting	1
80	NCT05812326—China	MUC1-positive breast cancer	MUC1-specific CAR-T Cells	Anti- MUC1 insertion & both TRAC & PD-1 knockout	CRISPR/Cas9—ex vivo	17/5/2019	1/2
81	NCT05037669—not pro-vided	Relapsed/refractory CD19 + leukemia and lym-phoma	CD19-specific CAR-T cells	Anti-CD19 insertion & TRAC, B2M & CIITA knockout	CRISPR/Cas9—ex vivo	7/2022—withdrawn	1
82	NCT03398967—China	Relapsed/refractory leuke-mia and lymphoma	Dual Specificity CD19 & CD20 or CD22 CAR-T cells	Combined anti-CD19 & anti-CD20 or anti-CD19 & anti-CD22 insertion & TRAC knockout	CRISPR/Cas9—ex vivo	2/1/2018—unknown	1/2
83	NCT05397184—UK	T Cell Malignancies	CAR-T cells	Modification of T cell genome	Base editing—ex vivo	19/4/2022—recruiting	1

Data recruited from the Clinical trials website (https://clinicaltrials.gov) by September 9, 2023. The table includes the interventional clinical trials only, in which participants receive one or more interventions. NA: not applicable; AML: acute myeloid leukemia; EBV: Epstein-Barr virus; CAR-T cells: chimeric antigen receptor T cells; CBE: cytosine base editor; CCR5: C–C chemokine receptor 5; CHL: congenital hearing loss; CISH: cytokine inducible SH2 containing protein; CRISPR/Cas9: clustered regularly interspaced short palindromic repeats/CRISPR-associated enzyme 9; CTL: specific cytotoxic T lymphocytes; DMD: Duchenne muscular dystrophy; HAE-3: hereditary angioedema type 3; HeFH: heterozygous familial hypercholesterolemia; HIV: immunodeficiency virus; HPV: human papillomavirus; HSCs: hematopoietic stem cells; HSPCs: hematopoietic stem/progenitor cells; I2S: iduronate 2-sulfatase; IDUA: alpha-L-iduronidase; iHSCs: induced-HSCs; LCA10: leber congenital amaurosis 10; MPS: mucopolysaccharidosis; OTOF: otoferlin; PAH: phenylalanine hydroxylase; PCSK9: proprotein convertase subtilisin/kexin type 9; PD-1: programmed death receptor 1; RHO: rhodopsin; PKU: phenylketonuria; RP: retinitis pigmentosa; SCD: sickle cell disease; T1D; type 1 diabetes mellitus; TALEN: transcription activator-like effector nuclease; TDT: transfusion-dependent β-thalassemia; UK: United Kingdom; US: United States; ZFN: zinc finger nuclease

on monogenic disorders will exponentially increase in the upcoming few years. Four of these trials were designated to insert a correct copy of the defective gene in hemophilia B, phenylketonuria (PKU), and mucopolysaccharidosis type I and type II (MPSI and MPSII). Moreover, three aimed to restore the most common genetic mutations in Duchenne muscular dystrophy (DMD), leber congenital amaurosis 10 (LCA10), and otoferlin (*OTFO*)—related congenital hearing loss (CHL).

Alternatively, disruption of a specific target gene has been the leading mechanism in three other studies involving heterozygous familial hypercholesterolemia (HeFH), hereditary angioedema type 3 (HAE-3) and rhodopsin (*RHO*)—related retinitis pigmentosa (RP). 25% of autosomal dominant RP is associated with *RHO* mutations mediating retinal degeneration via gain-of-function effects in the vast majority of cases. Therefore, the therapeutic regimen should provide specific inhibition of mutant allele expression. An alternative approach involves disrupting both mutant and wild-type (WT) alleles and replacing them with an exogenous correct gene copy [49]. In the clinical trials of HeFH and HAE, the target genes (*PCSK9* and *KLKB1*) are not the mutant ones. HeFH is primarily caused by heterozygous mutations in the LDL receptor (*LDLR*) gene, reducing LDL cellular intake. In turn, the level of blood LDL will be elevated, possibly causing cardiovascular disease. PCSK9 is the negative regulator of LDLR, promoting its degradation, and *PCSK9* knockout was reported to increase *LDLR* gene expression in cases of HeFH [50]. HAE-3 is a rare genetic disorder characterized by recurrent, severe, and possibly fatal swelling attacks that affect different tissues and organs. It can originate due to mutations in the *F12* gene encoding for coagulation factor XII, which is a critical stimulator for blood coagulation and body inflammation via the intrinsic coagulation pathway and the kinin-kallikrein system, respectively. Kallikrein is encoded by the *KLKB1* gene in a protein precursor form (prekallikrein), where *KLKB1* disruption could ameliorate the disease state [51].

b. Diabetes mellitus

Two clinical trials have been established in Canada for treating type 1 diabetes mellitus (T1D), in which allogeneic pancreatic endoderm cells were genetically modified to promote immune evasion, preventing host rejection upon transplantation.

c. Viral infections

Most clinical trials on viral infections are related to the immunodeficiency virus (HIV), which can destroy the body's immune system, causing acquired immunodeficiency syndrome (AIDS). The first HIV clinical study was started in 2009, representing the pioneering gene editing-based study. To date, there are 11 HIV-related trials that are mainly disrupting the gene encoding the C–C chemokine receptor 5 (CCR5) in T cells or hematopoietic stem/progenitor cells (HSPCs). CCR5 is the key co-receptor for viral entry, mediating a fundamental role in HIV infection. Individuals with a 32-bp deletion in *CCR5* are naturally resistant to HIV infection. [52]. Alternatively, one study has been designed to eliminate large sections of the HIV genome, minimizing virus activity.

Other clinical studies on viral infections include coronavirus disease 2019 (COVID-19), herpes simplex virus type 1 (HSV1), and papillomavirus (HPV). The serious pandemic of COVID-19 has engaged many scientists to find an effective intervention to inhibit or treat the viral infection. COVID-19 is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The angiotensin-converting enzyme 2 (ACE2) is a surface protein on many cell types that allows SARS-CoV-2 to infect human cells [53]. On the other hand, the expression of programmed death receptor 1 (PD-1) has been reported to be significantly increased on the surface of T cells in patients with COVID-19. Continuous PD-1 expression causes T cell exhaustion, which in turn may reduce the patient's ability to fight reinfection [54]. Accordingly, a clinical trial was conducted for the knockout of both *PD-1* and *ACE2* in memory T cells to test the engineered cells ability for virus recognition and destruction upon reinfection.

Corneal infection with HSV1 induces herpes stromal keratitis (HSK), which is the major cause of infectious blindness [55]. A clinical trial has been designed to combat HSV1 in corneal tissue cells via the destruction of the viral genes UL8 and UL29 that are required for viral replication. On the other hand, HPV infection is the primary cause of cervical cancer. The viral E6 and E7 proteins disrupt the host tumor suppressor genes p53 and pRB, respectively, promoting cervical malignant transformation [56]. In this context, three clinical studies have been applied for E6 and/or E7 knockout in cervical neoplasms to abrogate viral activity.

d. Cancer

Notably, most gene editing-based clinical trials have been directed against different forms of malignant transformations. Programmed cell death protein 1 (PD-1) is a protein on the surface of T and B cells that down-regulates the immune system. PD-1 can prevent the progression of autoimmune diseases; however, it can also prohibit the immune system from eradicatingcancerous cells and tissues. Therefore, knockout of *PD-1* has been accredited by several clinical trials for various types of cancer ($n=7$) [57]. Moreover, PD-1 expression was found to be reduced

upon deficiency of cytokine-inducible SH2-containing protein (CISH) in T cells [58], justifying *CISH* knockout in other clinical trials ($n=2$).

Anti-cancer therapeutic strategies have been advanced by the development of T cell receptor (TCR)-based therapy. Generally, there are two types of TCR: alpha/beta (α/β; encoded by *TRAC* and *TRBC*, respectively) and gamma/delta (γ/δ; encoded by *TRGC* and *TRDC*, respectively), both of which are composed of a heterodimer and associated with invariant CD3 complexes on the cell surface. α/β TCR-expressing T cells comprise the most predominant T cell fraction, and they recognize antigens presented by major histocompatibility complex (MHC) molecules [59]. In TCR therapy, T cells are engineered with receptors targeting specific antigens of cancer cells. Another type of genetically modified T cell is the chimeric antigen receptor (CAR) T cell. They are engineered to produce synthetic CAR receptors on their surface. Each CAR bridges the cell membrane, where its extracellular part recognizes and binds specific antigens on cancer cells [60]. The main difference between TCR and CAR T cell therapies is the type of programmed receptor. In TCR therapy, the receptor binds antigen presented by MHC. However, CAR T cells bind naturally occurring antigens on the surface of cancer cells.

It is costly and time-consuming to engineer T cells per each patient. Autologous T cell therapy can also be hindered by the reduced quantity and quality of autologous T cells as patients usually receive lympho-depleting chemo- and/or radiotherapy. In this context, utilization of allogeneic universal T cells would provide an effective avenue for cancer treatment, in which T cells derived from healthy donors, engineered to express anti-cancer receptor (to recognize and destroy cancer cells) with simultaneous elimination of the TCR, specifically TRAC (to avoid graft-versus-host disease; GVHD) [61].

Islamic ethical perspectives on human gene editing-based studies

The Islamic discipline of bioethics is dominated by the collective reasoning of both religious scholars and biomedical scientists. In this context, three authoritative institutions have already been established, namely the Islamic Organization for Medical Sciences (IOMS), the Islamic Fiqh Academy (IFA), and the International Islamic Fiqh Academy (IIFA).

According to the Islamic ethical perspective, the field of genomics should generally comply with two main principles. First, the marriage institution is the only channel through which children can be procreated. Second, the research must firmly respect human dignity, i.e., informed consent should be gained, privacy should be highly appreciated, and the key benefits and harms should be rigorously evaluated. On the other hand, the Islamic ethical judgment on gene editing is specifically based on the purpose of editing (research, treatment, or enhancement) and the type of edited cells (somatic or germline).

Gene editing for research and treatment purposes is permissive. However, the three Muslin bioethics institutions prohibit gene editing for enhancement. They consider the latter act as tampering with God's creation. In this context, somatic gene editing is permitted only for research and treatment but not for enhancement (e.g., increasing musculature can be authorized in a patient with muscular atrophy but not in a person with normal capacities). However, germline gene editing is currently acceptable for research purposes only, where surplus or nonviable embryos obtained from in vitro fertilization can be utilized [62].

Conclusion

Different approaches of gene editing have been engineered and applied with the superior advantages of CRISPR-Cas systems by virtue of their high efficiency, simple design, and diverse manipulations. Extensive efforts are being made to enhance the technology efficacy, and it is anticipated that gene editing technology will eventually realize its great potential for effective disease amelioration and treatment. The profound therapeutic prospect for gene editing on diverse human disorders will prioritize its application in the near future.

Abbreviations

ABEs	Adenine base editors
CBE	Cytosine base editor
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats/CRISPR-associated enzyme 9
dCas9	Dead Cas9
DSBs	Double-strand breaks
HDR	Homology-directed repair
HSCs	Hematopoietic stem cells
NHEJ	Nonhomologous end joining
PEs	Prime-editors
pegRNA	Prime-editing guide RNA
PNAs	Peptide nucleic acids
RT	Reverse transcriptase
SSBs	Single-strand breaks
tcPNA	Tail-clamp PNA
TALEN	Transcription activator-like effector nuclease
UGI	Uracil DNA glycosylase inhibitor
ZFN	Zinc finger nuclease

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