A new age of precision gene therapy

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Gene therapy has become a clinical reality as market-approved advanced therapy medicinal products for the treatment of distinct monogenetic diseases and B-cell malignancies. This Therapeutic Review aims to explain how progress in genome editing technologies offers the possibility to expand both therapeutic options and the types of diseases that will become treatable. To frame these impressive advances in the context of modern medicine, we incorporate examples from human clinical trials into our discussion on how genome editing will complement currently available strategies in gene therapy, which still mainly rely on gene addition strategies. Furthermore, safety considerations and ethical implications, including the issue of accessibility, are addressed as these crucial parameters will define the impact that gene therapy in general and genome editing in particular will have on how we treat patients in the near future.

Introduction

The human body is composed of approximately 4×10^{13} cells, each fulfilling predefined roles to keep us alive and fully functional.[1] Information that directs cell function and fate is found in the coding and non-coding regions of our genome, located mainly in the cell nucleus (with additional information stored in the mitochondria). Genes, the units of coding information, get copied and trimmed before serving as mRNA for protein production. These processes of transcription and translation are highly regulated. Changes to the genome might result in the gain or loss of a gene's function, altered gene regulation, or changes in the amount of a given gene product. These changes can affect the survival of cells or affect organ function and, thus, the health of the individual.

Instead of treating symptoms, gene therapy strives to directly address the genetic drivers of the disease (figure 1).[2] Initial approaches for the treatment of monogenetic diseases focused on strategies that replaced a missing gene function through the ex vivo or in vivo transfer of genetic information into a patient's cells. These gene addition therapies became part of medical care on Oct 25, 2012, when the European Commission, following recommendation from the European Medicines Agency, approved the use of alipogene tiparvovec for patients with lipoprotein lipase deficiency.[3] At the time of this Therapeutic Review, the US Food and Drug Administration (FDA) or European Commission have approved 11 further gene addition therapies to treat monogenetic diseases, namely severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID), inherited retinal dystrophy caused by *RPE65* gene mutations, β-thalassaemia, spinal muscular atrophy type I, metachromatic leukodystrophy, early cerebral adrenoleukodystrophy, haemophilia A, haemophilia B, aromatic L-amino acid decarboxylase, Duchenne muscular dystrophy, and *COL7A1*-deficient dystrophic epidermolysis bullosa.[4,5] These medical conditions are all considered rare, with no or few therapeutic options available through standard care. The above-mentioned gene addition therapies were developed in response to unmet medical needs and are clearly affecting the quality of life of patients, families, caretakers, and physicians.

Despite this success, gene addition therapies have their limitations. They are restricted to recessive monogenetic diseases and face the challenge of fine-tuning the activity of the additional gene copy. Gene expression is mainly regulated by promoter and enhancer sequences. Although they are preferred, native promoters and enhancers are often too large to be used, given the restricted cargo capacity of current delivery systems. Moreover, besides promoter and enhancer sequences, genome structure, neighbouring coding and non-coding sequences, and the actual location within the nucleus are factors that influence the level of expression of a given gene.[6] These factors are insufficiently controlled by gene addition therapy.

This Therapeutic Review aims to provide an overview of genome editing technologies as a new and upcoming option for treating diseases. This Therapeutic Review includes a critical appraisal of the data collected and a discussion about challenges and next steps and how they can be applied to overcome some of the above-mentioned limitations, thereby expanding both the strategies that are applied in gene therapy and the disease areas that can be addressed. For example, since genome editing enables the direct repair of disease-

Search strategy and selection criteria

We searched MEDLINE, PubMed, and ClinicalTrials.gov for articles and published clinical trials concerning gene therapy and genome editing with the search terms "gene therapy", "genome editing", "designer nucleases", "CRISPR-Cas9", "zinc finger nucleases", "TALENs", "meganucleases", "base editors", "prime editors", "epigenome editing", "non-viral vectors", "viral vectors", and "safety assessment". The number of human clinical trials stated in the main text is based on the results for the search term "Edited OR CRISPR" in ClinicalTrials.gov only. Of the 225 search results published before Aug 14, 2023, only roughly a third (including 3 withdrawn studies) were genome editing studies. We focused on translational and clinical work related to inherited and acquired diseases that are treatable with gene therapy. Due to space limitations and the large number of publications in these fields, it was not possible to reference every relevant publication.

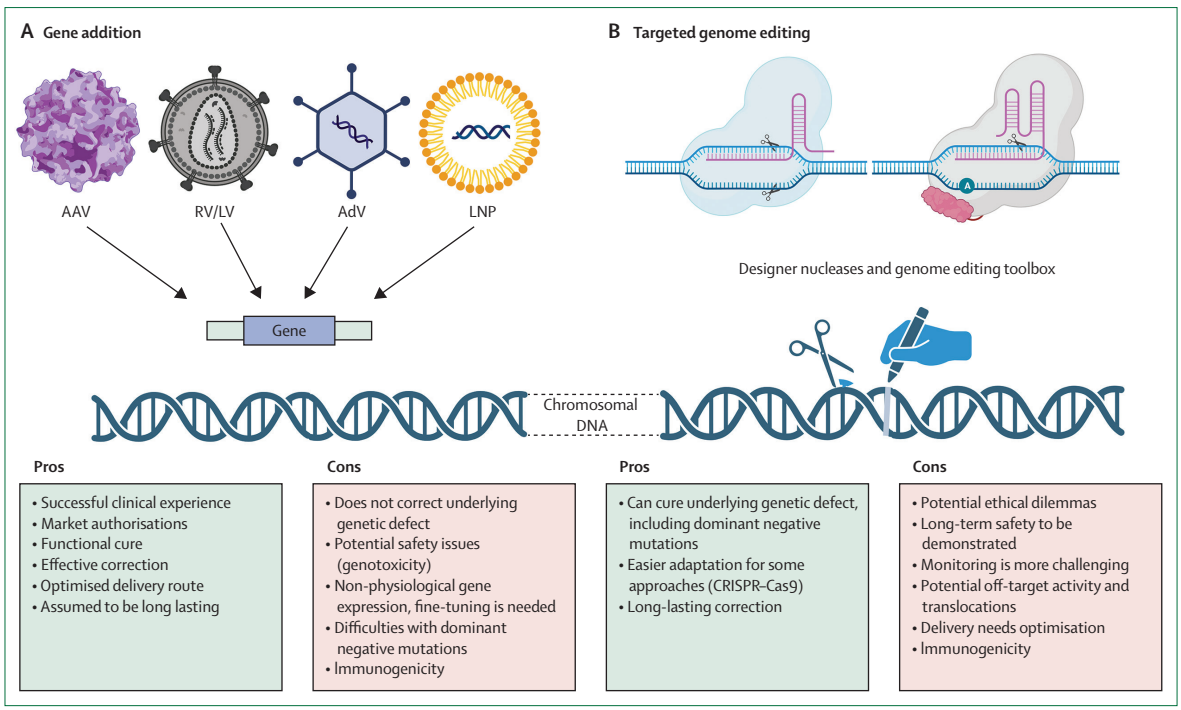

Figure 1: **Gene addition approaches versus targeted genome editing approaches**
(A) During gene addition, viral vectors, including AAV vectors, RV/LV vectors, AdV vectors, and non-viral vectors (eg, LNPs), deliver a whole gene of interest with promoter or enhancer elements and polyadenylation signals. (B) Designer nucleases and the described genome editing toolbox (eg, based on CRISPR–Cas9) lead to targeted gene editing with defined nucleotide changes in the genome. AAV=adeno-associated virus. AdV=adenoviral. Cas=CRISPR-associated protein. LNPs=lipid nanoparticles. RV/LV=lentiviral or gammaretroviral.

causing mutations, it provides a straightforward solution for dominant monogenetic diseases for which gene addition strategies—relying on the delivery of a wild-type copy of the mutated gene—would simply be ineffective. Furthermore, because the therapeutic target for genome editing approaches is the affected gene, it is located in its natural nuclear microenvironment with its natural regulatory circuits, a condition that cannot be mimicked by gene addition therapy as discussed earlier (figure 1). Thus, with the advance of gene editing to the clinical routine through the first market approval of a gene editing-based therapy on Nov 16, 2023,[7] gene therapy has entered a new age.

Genome editing tools and modes of action

The process of genome editing is initiated by introducing a DNA double-strand break (DSB) at a user-predefined target sequence. For this purpose, mainly engineered endonucleases are explored, which are termed designer nucleases to distinguish them from naturally occurring DNA-modifying enzymes. The DSB is sensed by the cellular DNA repair machinery and repaired by either the non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways (figure 2).

In NHEJ, the ends of the DSB are immediately ligated without proofreading for errors. Consequently, this process is prone to the introduction of insertions and deletions (also known as indels) that are likely to destroy the coding sequence of the targeted gene,[8] thus, NHEJ-based gene editing strategies are typically used to introduce gene knockout mutations. This is a clinically relevant strategy when targeting diseases caused by the overexpression of a gene, a gain-of-function mutation, or dominant negative mutations, which have all been reported, for example, for some forms of inherited retinal dystrophy.[9] In contrast, HDR is employed to precisely correct a mutation according to a DNA sequence, which is co-delivered with the designer nuclease and serves as a repair template. However, HDR-mediated repair relies on cellular proteins that are solely available in the G2 and S cell cycle phases, thus restricting its use mainly to proliferating cells.

Many designer nuclease tools have been developed in the past 15 years, with meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR–CRISPR-associated protein 9 (Cas9) system[10–13] as the most prominent examples (figure 2). First-generation meganucleases are naturally occurring endonucleases that were discovered in eukaryotic cells.[14] Since target sequences of meganucleases are rather long, as they encompass at least 20 nucleotides, meganucleases are known for their low off-target activities.[15] Besides naturally occurring meganucleases, synthetic meganucleases have been developed and are used as designer nucleases.[12] ZFNs rely on a set of zinc-finger proteins derived from

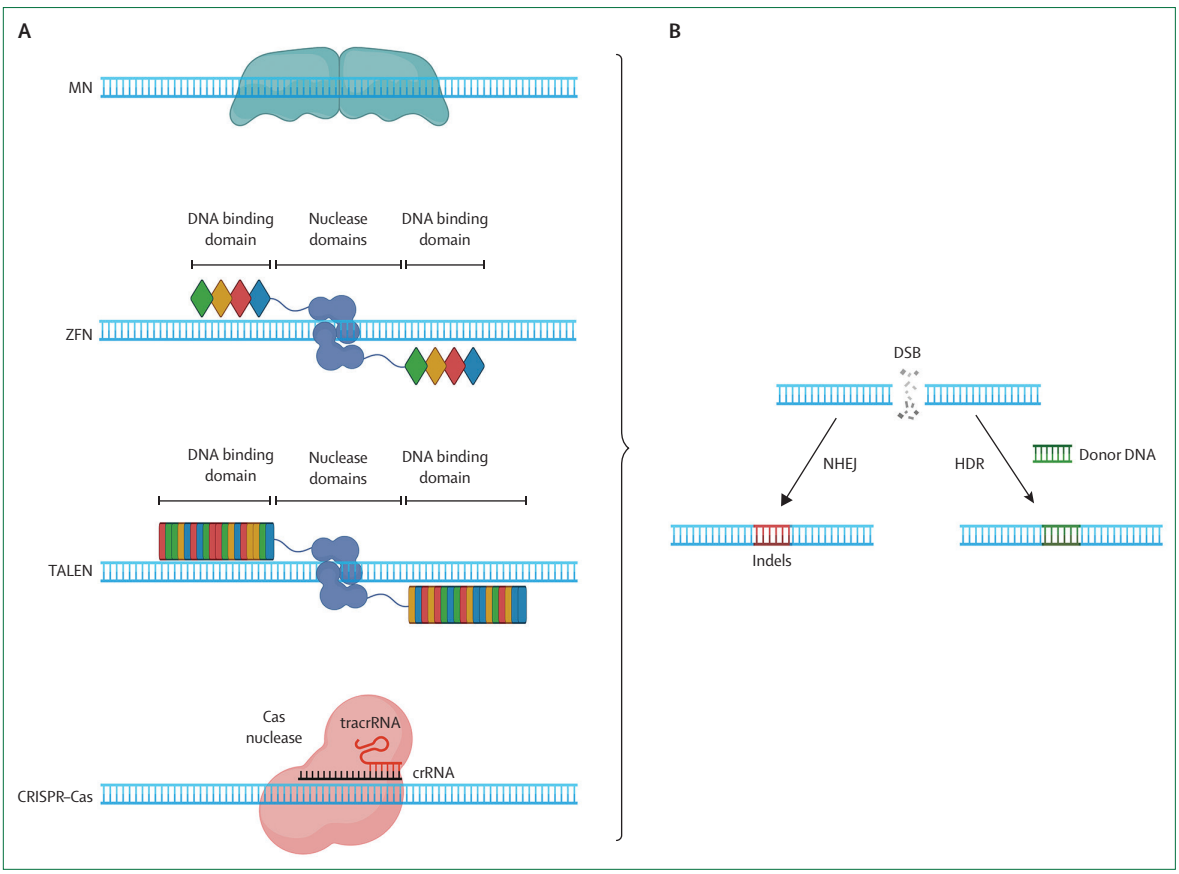

Figure 2: **The genome editing toolbox**
Distinct tools for genome editing have been developed over the years. (A) Schematic representation of the functional subunits of different genome editing systems. (B) Consequences of designer nuclease activity. Independently from their design, designer nucleases act by recognising specific DNA sequences, which are predefined by the user, and introducing DSBs that can be repaired by either the NHEJ or the HDR pathways. ZFNs and TALENs act as a pair, with one ZFN or TALEN binding to the user-predefined target sequence on the forward DNA strand and one binding to the reverse strand, to position their nuclease domain for introducing the DSB. Cas=CRISPR-associated protein. crRNA=CRISPR RNA. DSB=double-strand breaks. HDR=homology-directed repair. Indels=insertions and deletions. MN=meganuclease. NHEJ=non-homologous end joining. TALEN=transcription activator-like effector nuclease. tracrRNA=trans-activating crRNA. ZFN=zinc-finger nuclease.

eukaryotic transcription factors, which have been engineered to recognise user-predefined target DNA sequences in pairs. Each protein in a pair has been equipped with a nuclease domain, which needs to dimerise to cut DNA.[16,17] TALENs use the DNA-binding specificity of transcription activator-like effectors, proteins that are secreted by *Xanthomonas* bacteria to bind to promoter regions and reprogramme plant cell behaviour.[18] TALENs have also been engineered to contain a nuclease domain and to operate in pairs.

Since the DNA-binding domains of meganucleases, ZFNs, and TALENs are proteins, protein engineering techniques must be applied to direct the specificity of these tools to new DNA target sequences, which is a cumbersome and time-consuming procedure, as new proteins need to be engineered for every new target sequence. This issue is different in the case of the CRISPR–Cas9 system, in which the nuclease Cas9 is guided to the user-predefined DNA sequence by an RNA molecule, which is complementary to the target sequence. Changing the target sequence of an RNA molecule is a simple and easy task. Consequently, the CRISPR–Cas9 system has become the most versatile designer nuclease in use (figures 2, 3).

In nature, CRISPR–Cas9 serves as the adaptive immune system of bacteria and archaea to defend them against incoming phages.[19–21] Its specific DNA sequence recognition capacity was subsequently exploited for research and clinical purposes. The type II CRISPR–Cas systems are the most broadly used and contain only the Cas9 protein as a nuclease, which (in nature) is targeted and guided towards its DNA target sequence by a dual RNA molecule composed of the CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA).[21] To simplify the use of the system, crRNA and tracrRNA were combined into a single-guide RNA (sgRNA). Following positioning of the sgRNA-guided Cas9 protein at the target sequence, the DSB is introduced by the two nuclease domains of the Cas9 protein, each cleaving one of the two DNA strands (figure 3A).

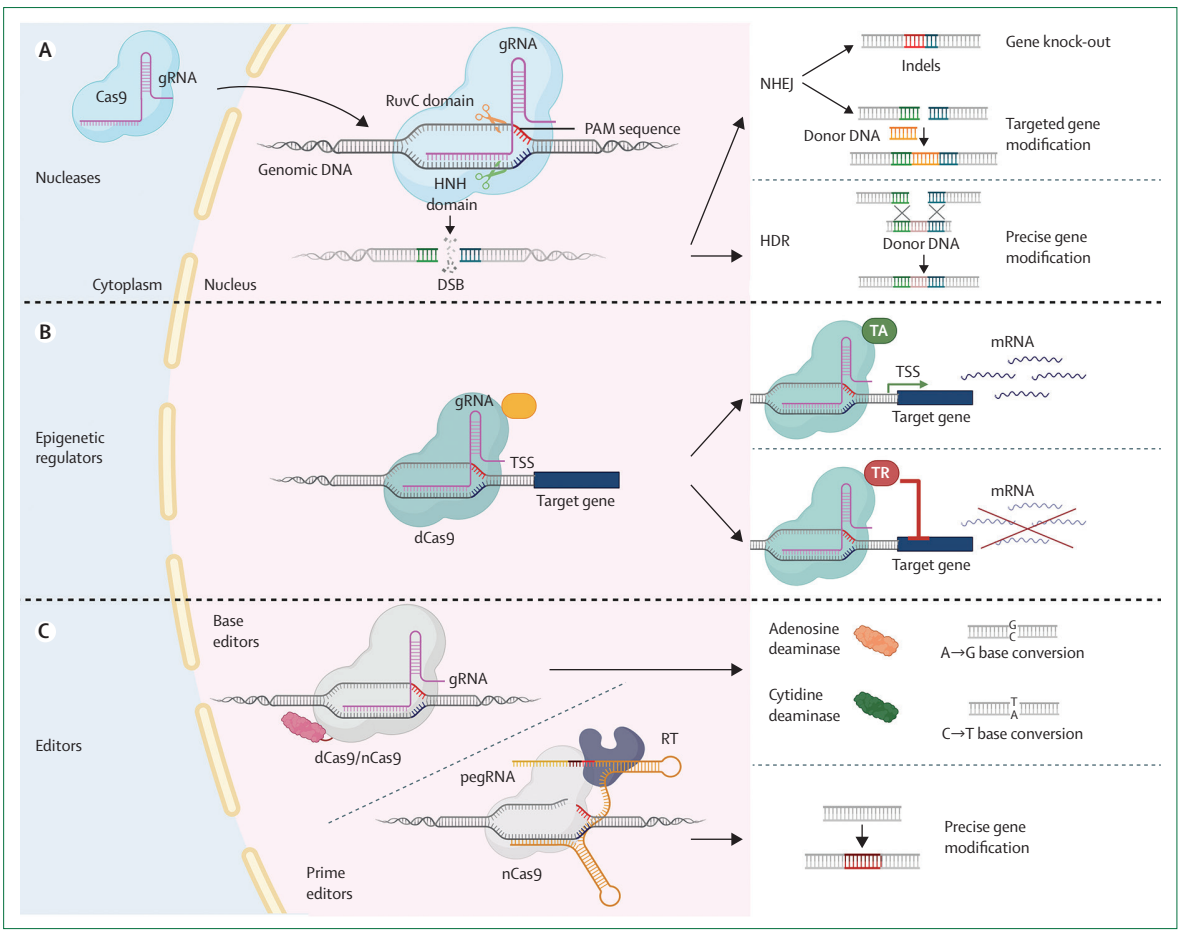

Figure 3: **Characteristics and mechanisms of CRISPR–Cas9-based gene editing tools**
Several types of CRISPR-associated nucleases are currently available along with different options for their delivery to cells. The direct delivery of Cas9-based approaches as RNP is used as an example. Alternatively, both the Cas9 protein and gRNA can be delivered as DNA/mRNA via either viral or non-viral vectors. (A) Once bound to the target strand of DNA, the Cas9 nuclease undergoes a conformational change, allowing for a targeted DSB in the genome, which can be repaired by different host cell repair machineries. NHEJ results in indels or the integration of an exogenous donor template. Alternatively, the host cell repairs the Cas9-induced DSB via the more precise HDR pathway. (B) Specific mutations in the Cas9 sequence have been explored to generate dCas or nCas. Although they lack the ability to generate DSBs, these Cas variants retain binding properties to DNA–gRNA hybrids and recognise specific target sites in the genome. Therefore, dCas or nCas versions have been repurposed as synthetic DNA binding platforms, which can be fused to specific effector domains to perform several distinct tasks at the target locus. They can, for example, be used to recruit epigenetic effectors (the yellow domain) that directly alter gene expression at specific loci. When fused to a TA, such as VPR or VP64, dCas9 recruits transcriptional agonists to promoters to increase expression of target genes. Alternatively, fusion of TSs, such as KRAB, to dCas9 interferes with target gene transcription, therefore knocking down gene expression. More advanced versions of dCas9-based platforms have also been developed to reorganise chromatin architecture. (C) Base editors are the fusion of dCas/nCas and a deaminase enzyme (with or without a DNA glycosylase inhibitor), which allow for the direct conversion of a single nucleotide. ABE converts an adenine and thymine base pair into a guanine and cytosine base pair; CBE converts a cytosine and guanine into a thymine and adenine base pair. As such, base editing can introduce all four transition mutations. Prime editors consist of an RT fused to nCas9 and a pegRNA. The pegRNA is a gRNA with an extension at the 3′ end of the scaffold that contains two additional functional domains: a primer-binding sequence (in light yellow) complementary to the 3′ end of the nicked DNA strand, and an upstream RNA sequence that serves as a template for reverse transcription. Upon Cas9-mediated generation of the nick, the 3′ flap of the nicked DNA forms a sequence-specific interaction with the primer-binding site in the pegRNA. This RNA–DNA hybrid serves as the primer site for the RT to synthesise new DNA with the genetic information included in the pegRNA template region, thereby extending the 3′ flap. This newly synthesised, edited DNA strand can displace the previously present DNA strand, leading to the formation of a DNA heteroduplex consisting of one edited and one unedited strand. The cellular repair systems can subsequently replace the original sequence with the edited sequence. Prime editors can perform all 12 possible transition and transversion mutations and small indel mutations. dCas=nuclease-null (or dead) Cas9 protein. DSB=double-stranded break. gRNA=guide RNA. HDR=homology-directed repair. Indels=insertions and deletions. nCas=catalytically impaired nuclease Cas9 nickase that cleaves only one of the two strands of DNA. NHEJ=non-homologous end joining. PAM=protospacer-adjacent motif. pegRNA=prime editing gRNA. RNP=ribonucleoprotein. RT=reverse transcriptase. TA=transcriptional activator domain. TS=transcriptional repression domain. TSS=transcriptional start site.

The introduction of mutations into one of the two Cas9 nuclease domains results in so-called nickases that cleave only one DNA strand, whereas mutating both domains generates a so-called dead or catalytically inactive Cas9 protein (dCas9 or nCas9). The latter is used to position protein effector domains, such as epigenetic modifiers, at defined target sequences to modify the transcriptional activity of a given target gene (figure 3B).[22,23] Fusing dCas9 or nCas9 proteins to a cytidine or adenine deaminase confers the capability of

Panel: Strategies to deliver designer nucleases

This panel provides an overview of the different strategies for delivering designer nucleases, or designer nucleases and their repair templates, to target cells following local or intravenous administration, and for overcoming the physical barrier of the cell membrane.

Physicochemical methods

Electroporation is a physicochemical method that causes transient destabilisation of the cell membrane. In this destabilised condition, nucleic acids, proteins, or complexes of nucleic acids and proteins (so-called ribonucleoprotein complexes) can cross the cell membrane and enter the cell. Electroporation is frequently applied in human clinical trials to transfer DNA or RNA encoding designer nucleases, designer nucleases as proteins, or CRISPR–Cas9 ribonucleoproteins into ex vivo cultures of haematopoietic stem cells or T cells, which are subsequently re-infused into patients.

Vector-based methods

Synthetic (non-viral) or virus-based vectors are used to transfer DNA or RNA encoding designer nucleases or the DNA repair template either to cells in culture (ex vivo) or directly to target cells or organs in patients (in vivo).

Lipid nanoparticles (LNPs) containing RNA as payloads are the most frequently applied non-viral or synthetic vectors in genome editing; the type and ratio of lipids in LNP formulations determine the physical particle features[28,29] and are crucial parameters for modulating the in vivo biodistribution of LNPs; in addition to the lipid formulation, strategies relying, for example, on the addition of targeting ligands, are under development to expand the spectrum of target cells and increase cell selectivity;[30] LNPs have fewer restrictions compared with viral vectors regarding cargo size and cargo type.[29]

The virus-based vectors, also termed viral vectors, applied in genome editing are mainly derived from adenoviral (AdV) vectors, adeno-associated viral (AAV) vectors, and integration-deficient lentiviral (IDLV) vectors;[31,32] these vectors either deliver DNA (AdV and AAV vectors) or RNA (IDLV vectors), exploiting evolutionarily optimised viral strategies to reach a cell, be internalised, and shuttle their genetic payload to the cell nucleus; all of these viral vectors share the common trait that vector genomes are maintained as episomes in the cell nucleus to limit the duration of their presence in proliferating cells and reduce the risk of insertional mutagenesis;[33] as mentioned for LNPs, targeting strategies enabling delivery to the therapy-relevant cell type in vivo are well advanced for all of these vectors;[34–36] notably for in vivo genome editing, mainly AAV vectors, with only a few exceptions (eg, NCT04560790), were employed in human clinical trials.

Combined strategies

Physicochemical methods are combined with vector-based methods, and combinations of different vector-based strategies are being explored. For example, electroporation was combined with viral vector-mediated gene addition approaches to improve function of genetically engineered T cells in cancer immunotherapy[37–40] or to restrict the presence of designer nucleases in target cells while efficiently providing the homology-directed repair template for ex vivo genome editing of haematopoietic stem cells.[41]

introducing precise nucleotide exchanges[24–26] to repair or modify genes (figure 3C)—termed base editing. To achieve a targeted insertion, deletion, or exchange of a DNA sequence in the absence of a DSB, prime editing was developed by combining a Cas9 nickase and a specialised RNA molecule known as a prime editing guide RNA (figure 3C).[27] All designer nucleases are large biomolecules for which the cell membrane is a natural barrier. Therefore, various delivery strategies have been developed (panel).[42] Strategies used in current human clinical trials are summarised in tables 1 and 2.

Applications of genome editing in human clinical trials

Until Nov 16, 2023, genome editing-based approaches were only investigated in human clinical trials. This situation changed with the first market approval in the world by the UK Medicines and Healthcare products Regulatory Agency (MHRA) for the treatment of individuals with sickle cell disease and transfusion-dependent β-thalassaemia (exagamglogene autotemcel).[7] Market approvals of this gene therapy in other countries are expected soon.[7] Of the more than 65 genome editing-based human clinical studies listed in the ClinicalTrials.gov database (as of August, 2023), 80% make use of ex vivo genome editing. The following examples reveal the breadth of these applications.

Improving T-cell function for cancer immunotherapy through ex vivo genome editing

Adoptive T-cell therapy with T cells modified with chimeric antigen receptors (CARs) directed against CD19 or B-cell maturation antigen (BCMA) has become the second-line treatment option for patients with CD19$^+$ B-cell lymphoma or leukaemia and the third-line treatment option for patients with BCMA$^+$ multiple myeloma.[4,5] To further improve CAR T-cell therapy, additional modifications can be introduced with genome editing. For example, genome editing is used to eliminate self-target antigens from CAR T cells, thereby addressing the challenge of fratricide, ie, CAR T cells killing other CAR T cells when designed to target the T-cell markers CD5 or CD7 to treat T-cell lymphomas.[38] Similarly, genome editing is used to

	Status	Genome editing system	Indication	Therapeutic gene	Genome editing transfer	Phase	Enrolled patients (n)*	Targeted gene and cell	Sponsor	Country	Notes and references
NCT02388594	Completed	ZFN nuclease	HIV	NA	Electroporation of ZFN mRNA	1	14	KO of *CCR5* in autologous T cells	University of Pennsylvania, NIAID, and Sangamo Therapeutics	USA	Tebas et al (2021)[43] and seven similar studies listed on ClinicalTrials.gov
NCT02793856	Completed	CRISPR–Cas9	NSC lung cancer	NA	Electroporation of plasmids	1	12	KO of PD-1 in autologous T cells	Sichuan University and Chengdu MedGenCell	China	Study reported in Lu et al (2020)[44] and commentary in He (2020)[45]
NCT05566223; NCT04426669	Ongoing	CRISPR–Cas9	NSC lung and gastrointestinal cancer	NA	Electroporation of RNA	1 and 2	70; 20	KO of *CISH* in TILs	Intima Bioscience	USA	Palmer et al (2022);[46] Palmer et al (2015);[47] and Elisa et al (2021)[48]
NCT04976218	Ongoing	CRISPR–Cas9	Solid tumours	*EGFR-CAR*	Electroporation of RNA	1	30	KO of TGFβ receptor-2 in CAR T cells	Chinese PLA General Hospital	China	..
NCT04037566	Ongoing	CRISPR–Cas9	Leukaemia or lymphoma	*CD19-CAR*	Electroporation of RNA	1	40	KO of *HPK1* in autologous T cells	Xijing Hospital and Xi'An Yufan Biotechnology	China	HPK1 is a negative regulator in T cells (Sawasdikosol and Burakoff, 2020);[49] study referenced in Basar et al (2020)[50]
NCT04637763	Ongoing	CRISPR–Cas9	B-cell lymphoma	*CD19-CAR*	Electroporation; hybrid RNA–DNA guides AAV6 for HDR donor	1	72	KO of PD-1 in allogeneic T cell; KI of CAR in *TRAC* locus	Caribou Biosciences	USA	O'Brien et al (2022)[51] and Caribou Biosciences (2023)[52]
NCT02746952	Completed	TALEN	B-cell leukaemia	*CD19-CAR*	Electroporation of TALEN mRNA	1	25	KO of *CD52* and *TRAC* in donor T cells	Institut de Recherches Internationales Servier	USA, UK, EU, and Japan	Study results in Benjamin et al (2022)[53]
NCT05397184	Ongoing	Base editor	T-cell leukaemia	*CD7-CAR*	Electroporation of RNA	1	10	KO of *CD7* in allogeneic T cells	University College London	UK	Georgiadis et al (2021)[38] and Chiesa et al (2023)[54]
NCT04767308	Ongoing	CRISPR–Cas9	Lymphomas	*CD5-CAR*	Electroporation	1	18	KO of *CD5* in autologous T cells	Huazhong University of Science and Technology and Shanghai IASO Biotechnology	China	Strategy described in Dai et al (2021)[55]
NCT04849910	Ongoing	CRISPR–Cas9	Leukaemia	NA	Electroporation	1 and 2	18	KO of *CD33* in allogeneic HSC	Vor Biopharma	USA and Canada	Cooper et al (2022)[56] and the first study results in Koehne et al (2023)[57]
NCT05169489	Ongoing	MegaTAL	Non-Hodgkin lymphoma	*CD79α-CAR* and *CD20-CAR*	Electroporation of mRNA	1 and 2	50	KO of *CBLB* in autologous T cells	2seventy bio	USA	Locke et al (2022)[58]
NCT04502446; NCT04438083	Ongoing	CRISPR–Cas9	T-cell lymphoma; renal cancer	*CD70-CAR*	Electroporation	1	45; 107	Editing of *TRAC*, *B2M*, and *CD70* in allogeneic T cells	CRISPR Therapeutics	USA, Australia, and Canada; USA, Canada, Auustralia, and Netherlands	Iyer et al (2022)[59] and first study results in Sumanta et al (2022)[60]
NCT04244656	Ongoing	CRISPR–Cas9	Multiple myeloma	*BCMA-CAR*	Electroporation	1	26	KO of TCR and MHCI in allogeneic T cells	CRISPR Therapeutics	USA, Australia, Canada, and Spain	Dar et al (2018)[61]
NCT05456880	Ongoing	Base editor	Sickle cell disease	NA	Electroporation	1 and 2	15	Correction to increase HbF in HSC	Beam Therapeutics	USA	First patient enrolled in November 2022; Johnson (2022)[62]

(Table 1 continues on next page)

	Status	Genome editing system	Indication	Therapeutic gene	Genome editing transfer	Phase	Enrolled patients (n)*	Targeted gene and cell	Sponsor	Country	Notes and references
(Continued from previous page)											
NCT03745287; NCT03655678	Ongoing	CRISPR–Cas9	Sickle cell disease and β-thalassaemia	NA	Electroporation of RNP	1, 2, and 3	45; 45	Erythroid-lineage-specific enhancer of *BCL11A* in HSC	Vertex Pharmaceuticals	USA, EU, Canada, and UK	First results published in Frangoul et al (2021);[63] Frangoul et al (2022);[64] and Locatelli et al (2022)[65]
NCT04853576	Ongoing	CRISPR–Cas (AsCas12a)	Sickle cell disease	NA	Electroporation of RNP	1 and 2	40	Editing the promoter region of gamma globin genes 1 and 2 to increase HbF	Editas Medicine	USA and Canada	First results in Editas Medicine (2022);[66] and update in Johnson (2023)[67]
NCT04211480	Ongoing	CRISPR–Cas9	β-thalassaemia	NA	Electroporation of Cas9 RNP	1 and 2	6	Erythroid-lineage-specific enhancer of *BCL11A* in HSC	Bioray Laboratories	China	First results published in Fu et al (2022)[68]
NCT05340426	Ongoing	CRISPR–Cas9	Kidney failure	NA	Genome editing in pig cells	1	20	Disruption of four antigens in porcine germline	University of Alabama at Birmingham and Lung Biotechnology (United Therapeutics)	USA	Xenotransplantation; Porrett et al (2022)[69]
NCT05210530	Completed	CRISPR–Cas9	Diabetes	NA	Electroporation	1	10	Allogeneic pancreatic progenitor cells genetically modified for immune evasion and survival	CRISPR Therapeutics and Viacyte	Canada	Philippidis (2022)[70]
NCT04990557	Ongoing	CRISPR–Cas9	COVID-19	NA	Delivery of plasmids	1 and 2	16	KO of PD-1 and *ACE2* in autologous T cells	Academic	Egypt	..

AAV=adeno-associated virus. BCMA=B-cell maturation antigen. CAR=chimeric antigen receptor. Cas=CRISPR-associated protein. HbF=fetal haemoglobin. HDR=homology-directed repair. HSC=haematopoietic stem cells. KI=knock-in. KO=knock-out. MegaTAL=meganuclease–transcription activator-like repeats fusion protein. NA=not applicable. NIAID=National Institute of Allergy and Infectious Diseases. NSC=non-small cell. PD-1=programmed death protein 1. PLA=People's Liberation Army. RNP=ribonucleoprotein. TALEN=transcription activator-like effector nucleases. TILs=tumour-infiltrating T cells . ZFN=zinc-finger nuclease. *As given on ClinicalTrials.gov (actual n for completed studies, estimated n for ongoing studies).

Table 1: **Selected clinical trials for ex vivo genome editing**

inactivate molecules targeted by a co-administered drug, which are also expressed on CAR T cells. For example, inactivation of the glucocorticoid receptor on CAR T cells with ZFNs prevented its interference with CAR T-cell activities in a combination therapy for glioblastoma that used an interleukin-13 receptor-specific CAR along with glucocorticoids.[37]

Improvement of T-cell activity is the most common reason for applying genome editing to CAR T cells, tumour-infiltrating T cells, or recombinant T-cell receptor (rTCR) T cells. This improvement can be achieved by inactivating components of the TCR, immune checkpoint inhibitors, or the E3 ubiquitin-protein ligase.[46,86] An example from 2020 refers to the treatment of three patients with refractory cancer.[39] Patients received T cells, which, in addition to coding for rTCRs specific for New York esophageal squamous cell carcinoma (also known as NY-ESO), had been subjected to genome editing to inactivate both endogenous TCR chain genes (TCRα and TCRβ) and the programmed death protein 1 (PD-1)-encoding gene to reduce the risk of TCR mispairing and to improve the antitumour activity of the engineered T-cell product.[39] Overall, the study revealed initial safety despite the introduction of some off-target mutations and chromosomal translocations around the edited sites. The latter are due to ligation of the DSB that occurred on different chromosomes via an error-prone DNA repair by NHEJ. Notably, edited T cells persisted in patients over several months.[39]

Genome editing is also of special relevance for off-the-shelf CAR T cells. Off-the-shelf CAR T cells

	Status	Genome editing system	Indication	Phase	Enrolled patients (n)	Targeted gene and organ	Genome editing transfer	Dose	Sponsor	Country	Notes and references
NCT05398029	Ongoing	Base editor	Familial hypercholesterolaemia	1	44	*PCSK9* in liver	RNA-LNP	..	Verve Therapeutics	New Zealand and UK	Preclinical data in Musunuru et al (2021)[71] and Lee et al (2023);[72] first patient dosed in 2022 (Verve Therapeutics);[73] and trial placed on hold by the FDA in November, 2022 (Verve Therapeutics)[74]
NCT05222178	Ongoing	Homologous recombination	Phenylketonuria	1	9	Correction of *PAH* gene in liver	AAVHSC15	..	Homology Medicines	USA	First patient dosed in 2023 (Standfield)[75] and first clinical updates in BioSpace (2023)[76]
NCT04601051	Ongoing	CRISPR–Cas9	Transthyretin amyloidosis	1	72	Inactivation of the *TTR* gene in liver	RNA-LNP	0·3 mg/kg	Intellia Therapeutics	UK, France, Sweden, and New Zealand	Results in Gillmore et al (2021)[77]
NCT03872479	Ongoing	CRISPR–Cas9	Leber congenital amaurosis (LCA10)	1 and 2	34	Correction of *CEP290* in retina	AAV5	..	Editas Medicine	USA	Preclinical data in Maeder et al (2019)[78] and clinical trial paused in 2022 (Johnson)[79]
NCT03041324	Completed	ZFN nuclease	Mucopolysaccharidosis type 2	1 and 2	9	*IDS* gene correction in liver	AAV6	5×10^{12} – 5×10^{13} vector genomes/kg	Sangamo Therapeutics	USA	Little robust clinical benefit reported on ClinicalTrials.gov and in Sangamo Therapeutics (2019)[80]
NCT02702115	Terminated	ZFN nuclease	Mucopolysaccharidosis type 1	1 and 2	3	Corrective copy of the *IDUA* transgene into liver	AAV6	..	Sangamo Therapeutics	USA	Ou et al (2020)[81] mention low efficacy
NCT04560790	Completed	CRISPR–Cas9	Viral keratitis	1	3	Inactivation of *HSV-1* gene in cornea	Lentiviral	..	Shanghai BDgene	China	..
NCT05120830	Ongoing	CRISPR–Cas9	Hereditary angioedema	1 and 2	55	Inactivation of *KLKB1* gene upon systemic administration	RNA-LNP	25–75 mg	Intellia Therapeutics	Netherlands, New Zealand, UK, France, and Germany	Intellia Therapeutics press releases with interim results on Sept 16, 2022,[82] on Nov 12, 2022,[83] and on March 2, 2023[84]
NCT05144386; NCT05143307	Ongoing	CRISPR–Cas9	HIV-1 infection	1	9	Excision of replication-competent proviral HIV	AAV9	..	Excision BioTherapeutics	USA	First patient dosed in 2022 (Balfour)[85]

AAV=adeno-associated virus. Cas=CRISPR-associated protein. FDA=US Food and Drug Administration. HSC=haematopoietic stem cells. LNP=lipid nanoparticles. ZFN=zinc-finger nuclease.

Table 2: **Selected clinical trials for in vivo genome editing**

are based on healthy donor-derived allogeneic T lymphocytes and were developed as an alternative option to autologous CAR T cells to reduce the time to treatment for patients and improve the quality and cell numbers of CAR T-cell transplants. To improve CAR expression, reduce the risk of alloreactivity, and mediate resistance against the CD52 monoclonal antibodies used for lymphodepletion, genome editing with TALENs was used to inactivate the *TRAC* and *CD52* genes in CAR T cells that expressed a CAR directed against the CD19 protein.[40] Results from a human clinical trial with 25 patients for these off-the-shelf, so-called universal CAR T cells have been reported.[53] 22 of the 25 patients (88%) received the lymphodepletion regimen with the CD52 antibody. 12 of the 25 patients exhibited a complete response and could be transferred to bone marrow transplantation. Long-term responses were reported for six patients. In 2022, off-the-shelf base-edited CAR T cells against $CD7^+$ T-cell acute lymphoblastic leukaemia were successfully used in a 13-year-old patient with refractory disease.[87] The patient remained in remission more than 10 months after the treatment.[87] Although these results are difficult to compare and are restricted to only a few patients, they suggest that off-the-shelf CAR T cells are safe and might indeed offer an alternative to the complex autologous CAR T-cell setting.

Ex vivo genome editing to develop innovative strategies against HIV infection

An HIV-positive patient with acute myeloid leukemia (known as the Berlin patient) who was treated in Berlin, Germany, received a bone marrow transplant from a donor whose cells had a deletion for the HIV coreceptor CCR5. After the transplantation, the patient's HIV levels dropped below the detection limit and remained undetectable, showing that T cells can be rendered resistant to HIV infection.[88,89] On the basis of this result, strategies are focusing on inactivating CCR5 in patients' T cells or haematopoietic stem cells with TALENs, ZFNs, or CRISPR-Cas9.[90] Although natural mutations in the CCR5 coreceptor can protect people from HIV infection, the clinical success of mimicking this strategy with genome editing has so far been limited to only a temporary benefit with a short-term selective advantage in the gene-edited cells. A 2021 trial used ZFNs to inactivate CCR5 in T cells and observed a delayed viral rebound and restoration of HIV-specific T-cell responses in a few patients.[43] Besides providing protection against HIV entry, strategies aiming to excise or inactivate the HIV provirus in latently infected T cells continue to be actively explored (eg, NCT05144386 and NCT05143307) and were among the first clinical applications of genome editing.[91]

Ex vivo genome editing strategies to modulate immune responses

Another strategy explored by genome editing is that of evoking immune evasion. For example, following genome editing, allogeneic pancreatic cells encapsulated in a perforated device were implanted into patients with type 1 diabetes to restore insulin production.[70] Genome editing is also being explored to enable the genetic engineering of pig organs for the purpose of xenotransplantation. The latter was developed as a potential solution to overcome the medical challenges associated with the poor availability of donor organs. However, prevention of immediate rejection is an even bigger challenge for organ xenotransplantation than for allogeneic human organ transplantation. In 2022, a patient with heart failure was xenotransplanted with a pig heart, a case that received broad public attention.[92] The patient's survival for 2 months can probably be seen as a success and demonstrates that the ten genetic modifications, including the inactivation of carbohydrate antigen genes, indeed prevented immediate rejection. More relevant information about the potential of xenotransplantation can be expected from future clinical trials. Notably, a first trial for patients with kidney failure will soon be initiated (NCT05340426).[69]

Ex vivo genome editing to expand treatment options in sickle cell disease and transfusion-dependent β-thalassaemia

Current clinical trials on ex vivo genome editing for monogenetic disease have a clear focus on sickle cell disease and transfusion-dependent β-thalassaemia. Sickle cell disease is caused by a distinct point mutation in the β-globin gene. The same gene is also affected in transfusion-dependent β-thalassaemia, but the mutations are far more heterogeneous. Instead of developing genome editing approaches to correct each of these mutations separately, an appealing alternative is to reactivate the expression of fetal globin genes, which offers the prospect of a single genome editing therapy for a diverse array of haemoglobinopathies, including sickle cell disease. A prime target of this approach is the transcription factor BCL11A, which is responsible for the decline of fetal globin levels after birth and subsequent increased β-globin levels.[93] Genome editing-based disruption of the erythroid enhancer controlling the *BCL11A* gene is used, resulting in fetal globin expression in patients with sickle cell disease and transfusion-dependent β-thalassaemia due to inactivation of the BCL11A transcription factor.[63] Results from seminal clinical studies[64,65] were presented in 2022: veno-occlusive crises were eliminated in all 31 patients with sickle cell disease (NCT03745287); transfusion dependency was eliminated in 42 of 44 patients who previously had transfusion-dependent β-thalassaemia (NCT03655678);[64] and stable editing and persistent fetal haemoglobin levels for more than 1 year post treatment were described.[65] On the basis of these results, the MHRA has granted a conditional market approval for this approach, which represents the first market approval for a gene editing-based approach in the world.[7] Similar strategies are being

followed in clinical trials that are just starting, which also include base editing of the *BCL11A* enhancer (NCT05456880) with investigational new drug clearance by the FDA. The first patient was treated in November, 2022.[94]

In vivo genome editing approaches with the liver as the target organ

A much broader variety of monogenetic diseases are tackled with in vivo genome editing (table 2). On systemic administration, lipid nanoparticles (LNPs) and viral vectors, including most adeno-associated viral (AAV) serotypes, distribute mainly to the liver. Accordingly, systemic delivery strategies for in vivo genome editing currently focus on diseases that can be addressed through gene inactivation in the liver.

One example is hereditary angioedema, a potentially fatal disease caused by mutations in the *KLKB1* gene that result in uncontrolled overexpression of kallikrein, which induces severe disseminated inflammations. According to a 2022 interim analysis of the sponsoring company of the ongoing study,[95] inactivation of the *KLKB1* gene through CRISPR–Cas9-mediated genome editing resulted in a more than 90% reduction of kallikrein levels in the high-dose patient cohort and strongly reduced rates of oedema events. In a related strategy, base editing is used to treat familial hypercholesterolaemia. The gain-of-function mutation in *PCSK9*, which codes for a protease responsible for degradation of LDL cholesterol receptors, is targeted for gene inactivation with systemically administered RNA–LNPs. This approach has shown impressive results in non-human primates[71] and a first patient was infused with this base-editing drug in late 2022 (NCT05398029).[96]

In vivo genome editing strategies have also been applied to treat phenylketonuria and the main forms of mucopolysaccharidosis; however, these studies were terminated due to little clinical benefit (table 2). The most promising example for in vivo genome editing is probably the treatment of transthyretin amyloidosis, a disease caused by the accumulation of misfolded transthyretin, which is a transport protein for the thyroid hormone thyroxine and retinol. The genetic defect results in a progressive fatal disease with polyneuropathy, and kidney and heart failure at an early age.[97] The therapeutic strategy involves the inactivation of the *TTR* gene by RNA-LNPs delivering Cas9 and guide RNA. As transthyretin is mainly produced in the liver and has few natural functions, the *TTR* gene appears to be ideally suited for disruption by in vivo genome editing. Results from the first-in-human clinical trial demonstrated substantial reductions of TTR serum levels of up to 90% in some patients.[77] Although these results show the high activity of the genome editing approach, the benefit for patients will largely depend on whether the deposited amyloid will be cleared over time once TTR levels remain low.

In vivo genome editing to expand treatment options in inherited retinal dystrophies

In settings in which organs other than the liver are the target, local vector administration into a secluded area is chosen. A prominent example is the eye. In vivo genome editing for eye-directed gene therapy mainly focuses on inherited retinal dystrophies, which affect more than 2 million people worldwide.[98] Inherited retinal dystrophies are genetically very heterogeneous, with more than 250 causative genes reported on the RetNet Retinal Information Network to date, often with multiple mutations in each gene. The potency of gene addition therapy for inherited retinal dystrophies was impressively demonstrated for Leber congenital amaurosis 2 (LCA2; caused by mutations in the *RPE65* gene), which led to the market authorisation of this treatment in 2018.[98] For other forms of inherited retinal dystrophies, such as LCA10 (which is due to mutations in the *CEP290* gene), the genes that cause the diseases are too large to be delivered by AAV vectors, the current gold standard in eye-directed gene therapy. In response, a genome editing strategy making use of the CRISPR–Cas9 system was developed to target the most common mutation in *CEP290*: IVS26 (2991+1655A→G), a point mutation located within an intron that creates a premature stop codon due to abnormal splicing.[78] A human clinical trial was initiated in 2020 (NCT03872479) and represents the first authorised investigation of the safety of CRISPR–Cas9 in humans (table 2). Another clinical study initiated in 2020 evaluated a CRISPR–Cas9 approach to inactivating two genes that are essential for the herpes simplex virus lifecycle as a strategy to reduce the virus load in three patients with severe refractory herpes stromal keratitis (NCT04560790). This single-arm trial showed proof of concept for the potential use of CRISPR–Cas9 genome editing approaches to treat human infectious diseases.[99]

For more on **RetNet** see https://web.sph.uth.edu/RetNet/

Safety considerations

One of the main concerns about genome editing relates to the question of how to reduce or better avoid the risk of unwanted effects,[100] such as modifications of the host genome at unintended sites (genomic off-target activity) or immune responses against designer nucleases. Since both the duration of the presence of designer nucleases in the patient or in the cells delivered to the patient and the applied dosage increases the treatment-associated risk, the use of CRISPR–Cas9 ribonucleoproteins (RNPs) or mRNAs encoding designer nucleases is preferred over DNA-based approaches because of the shorter half-life.[100] In cases when DNA formulations are used, self-inactivating strategies[101] are a possibility to prevent extended exposure to the nucleases. Likewise, controlled expression of the designer nuclease or, in the case of the CRISPR–Cas9 system, of crRNAs and sgRNAs showed promise.[102–107]

To improve risk assessment, off-target prediction assays are commonly used to increase the safety of genome editing approaches. To design the most effective and safe genome

editing applications, in silico or in vitro cell-free DNA off-target prediction assays are followed by cellular and genome-wide validation assays, such as GUIDE-Seq (which provides an unbiased identification of DSBs enabled by sequencing)[108] or CAST-Seq.[109] Among these and other safety testing strategies, methods were developed to find potential off-target sites; to find structural variations, including translocations,[110] chromothripsis,[111] or aneuploidy; and to identify unintended editing events.

As a further risk factor, it should be noted that the natural genetic diversity among individuals can potentially alter both the on-target and off-target outcomes of therapeutic genome editing and should be taken into consideration for sgRNA design.[112–114] For example, single nucleotide polymorphisms and small insertions and deletions cause substantial genetic variation in human genomes. In response, algorithms such as CRISPRme were developed to identify and prioritise off-target sites at both the population and the individual level, with the aim of helping to guide the development of safer, more effective genome editing strategies.[112] In addition, age-related and possibly disease-related clonal haematopoiesis could present additional challenges to genome editing strategies and should therefore be carefully considered.[115,116]

For more on **CRISPRme** see http://crisprme.di.univr.it/

Furthermore, to reduce the general risks associated with the generation of DSBs, regardless of whether they are introduced intentionally or unintentionally, genome editing strategies that do not rely on DSBs, such as base editing, prime editing, or the most recently developed CRISPR transposons, are being explored.[24–27,117,118] Moreover, to improve the safety of in vivo genome editing, cell-selective delivery tools[30,35,77,119–123] are being developed to reduce or possibly avoid the delivery of designer nucleases to non-target tissues or cell types.

The question of accessibility

An important concern to be addressed is the equitable distribution of these potentially life-saving therapies (see also statements from consortia[124,125] dealing with accessibility). Concerted efforts incorporating all major stakeholders and patient advocacy groups will be needed to reach this goal.[124–126] The organising committee of the Third International Summit on Human Genome Editing issued a statement in 2023[127] outlining the need for international collaboration around innovative approaches to regulate and develop genome editing technologies for cost-effective, affordable, and effective therapies. Cost-effectiveness is an important topic that needs to be considered as gene therapy applications are very expensive interventions.[128] Examples include patients with primary immunodeficiencies, whose estimated treatment costs for haematopoietic stem cell transplantations (HSCTs) are about US$400 000 versus $600 000 for ex vivo gene addition therapy for adenosine deaminase deficiency severe combined immunodeficiency.[129,130] Costs might be even higher for in vivo gene addition therapy for solid organ systems.[131] Although market approval for genome editing products remains to be obtained, we would expect that costs, at least for ex vivo approaches, will fall in between an HSCT and a conventional gene addition product, considering that the Good Manufacturing Practice (GMP)-grade ingredients for RNPs are likely to be less costly compared with GMP-grade viral vector preparations.

Regulatory guidance for the clinical use of genome editing

Concerted efforts are also required to fight misconduct. In the autumn of 2018, He Jiankui reported on the birth of twin girls whose genomes had been subjected to genome editing.[132] Although it has still not been proven that the genomes of the two girls were indeed modified, the announcement of such activity has already crossed the previously set boundaries agreed upon by scientists worldwide. Applying gene therapy to the cells of the germline is considered unethical, and scientists have agreed to abstain from such genetic manipulation even if tools and technologies become available. In some countries, suitable legal regulations prohibit germline modifications. With the current status of genome editing, not only efficiency but also safety considerations are additional practical barriers that need to be addressed beyond the clear ethical concerns surrounding germline modification.[133–135]

Genome editing-based therapeutics are regulated under the existing guidance available for advanced therapy medicinal products. The first genome editing-specific guidance document was released by the FDA in 2022. This document builds on existing recommendations for cell and gene therapy guidance documents and incorporates key considerations on the editing of human somatic cell genomes, including the information that needs to be provided in investigational new drug applications.[136] The guidance includes the thoughts of the Medicines Regulatory Agency on product design, manufacturing, preclinical safety testing, and clinical trials with human genome editing products. More detailed considerations, including in-depth discussion on the pros and cons of bioinformatic tools used to evaluate potential off-target sites, were published by a group of European regulators in 2022.[137] Together, both documents will serve as helpful guidance for applicants translating novel genome editing-based strategies into clinical trials.

Which diseases to treat

Possible examples of how gene editing could go beyond gene addition are discussed below.

Inherited retinal diseases as examples

Gene addition therapies show promise for some forms of inherited retinal diseases. However, in several common inherited retinal diseases, the therapeutic genes are too large to be delivered with a single AAV vector.[138] In response, genome editing-based approaches are being

explored to address this challenge, such as in the previously mentioned, ongoing human clinical trial for LCA10 (table 2). These approaches are considered as promising alternatives to strategies requiring specific designs to reconstruct a functional protein from vector genomes delivered by multiple AAV vectors that each encode parts of the gene.[138] Similarly, inherited retinal diseases that are caused by gain-of-function or dominant negative mutations in specific proteins[139] or in cases in which gene expression must be tightly regulated to be within a defined range for proper function are in need of genome editing-based strategies instead of gene addition strategies.

Liver-related diseases as examples

So far, two liver-directed gene addition therapies for the treatment of haemophilia A and haemophilia B have obtained market approval.[4,5] However, challenges in liver-directed gene therapy, such as the delivery of large genes, toxic gain-of-function mutations, and the safety or efficacy of expression from heterologous promoters, require genome editing as a possible solution. An additional major limitation of liver-directed AAV gene addition therapy is the episomal nature of AAV vector genomes, which results in their dilution in proliferating tissues, such as in newborn livers or adult livers undergoing regeneration in response to injury. Again, genome editing appears to be a promising strategy to tackle this issue, and gene knock-down, gene knock-in, and base-editing-based correction strategies are already under active development.[140]

Conclusion

Although we foresee a number of unique applications using genome editing (appendix p 2), genome editing is not expected to replace conventional gene addition therapy, and so both types of gene therapy will continue to be further developed. In contrast to gene addition, genome editing offers a potential medical intervention to cure the root cause of genetic disease through specific modification of the mutated genes, although several important challenges still need to be fully addressed. To realise the successful clinical translation and delivery of these promising therapeutic options to the patients who need them most, the continued support of structural organisations (including gene and cell therapy societies, medicines regulatory agencies, patient outreach groups and funding foundations) is crucial. Patients need to be informed of approved treatment options, and medical personnel need to be trained to administer and monitor patient outcomes with these new-age drugs. In regard to the question of which diseases to treat, diseases with a fatal and defined molecular diagnosis, but without available treatment options are considered as prime targets in gene therapy. Although the advent of genome editing has clearly expanded our options in this regard, the decision on whether to apply conventional gene addition therapy, genome editing, or a combination should be made on the basis of a thorough risk–benefit assessment.

Contributors

HB and AS developed the concept of this Therapeutic Review and defined the structural content. HB and AS organised the individual contributions and used these contributions to write the original draft. HB, AS, CJB, IT, RT-R, and MM analysed the underlying literature, contributed to the original draft, and edited and approved the final version of this Therapeutic Review. KC reviewed and edited the final version of this Therapeutic Review.

Declaration of interests

We declare no competing interests.

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See **Online** for appendix

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