The promise and challenge of therapeutic genome editing

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Genome editing, which involves the precise manipulation of cellular DNA sequences to alter cell fates and organism traits, has the potential to both improve our understanding of human genetics and cure genetic disease. Here I discuss the scientific, technical and ethical aspects of using CRISPR (clustered regularly interspaced short palindromic repeats) technology for therapeutic applications in humans, focusing on specific examples that highlight both opportunities and challenges. Genome editing is—or will soon be—in the clinic for several diseases, with more applications under development. The rapid pace of the field demands active efforts to ensure that this breakthrough technology is used responsibly to treat, cure and prevent genetic disease.

In the nearly seventy years since the discovery of the DNA double helix, technologies have advanced for the determination, analysis and alteration of genome sequences and gene-expression patterns in cells and organisms. These molecular tools are the foundation of molecular biology, driving the therapeutic industry by increasing the understanding of the genetics of normal and disease traits. The ability to diagnose genetic diseases has developed rapidly with reductions in the costs of genome sequencing, increases in comparative analyses of human genome sequences and increased applications of high-throughput genomic screening. However, the dearth of therapies, much less cures, for genetic diseases has created a growing separation between diagnostics and treatments, underscoring the urgent need to develop therapeutic options. Mitigation or correction of disease-causing mutations is a tantalizing goal with tremendous potential to save and improve lives, representing a convergence of technical and medical advances that could eventually eradicate many genetic diseases.

Although methods for genome engineering and gene therapy have been of interest for decades, the development of engineered and programmable enzymes for the manipulation of DNA sequences has driven a biotechnological revolution[1–5]. In particular, fundamental research showing how CRISPR and CRISPR-associated (Cas) proteins provide microorganisms with adaptive immunity has propelled transformative technological opportunities enabled by RNA-guided proteins. CRISPR–Cas9 and related enzymes have been used to manipulate the genomes of cultured and primary cells, animals and plants, vastly accelerating the pace of fundamental research and enabling breakthroughs in agriculture and synthetic biology[6–9]. Building on past gene therapy efforts[10], we are entering an era in which genome-editing tools will be used to inactivate or correct disease-causing genes in patients, offering life-saving cures to people who have genetic disorders.

In this Review, I discuss the therapeutic opportunities of genome editing, the ability to alter the DNA in cells and tissues in a site-specific manner. In addition to presenting current capabilities and limitations of the technology, I also describe what it will take to apply therapeutic genome editing in the real world. A comparison of somatic-cell and germline editing highlights the importance of open public discussion about, and regulation of, this powerful technology.

The scope of genome-editing applications

Although the genetics of human disease are often complex, some of the most common genetic disorders stem from mutations in a single gene. Cystic fibrosis, Huntington's chorea, Duchenne muscular dystrophy (DMD) and sickle cell anaemia each represent diseases that result from defects in only one gene in the human genome; such monogenic diseases, of which more than 5,000 are known, affect at least 250 million individuals globally. DNA sequencing of affected families has provided detailed information about the mutations that lead to each disorder, as well as correlations between specific genetic changes (genotype) and disease severity. These data in turn reveal DNA sequence alterations or corrections that could provide a genetic cure by either disrupting the function of a toxic or inhibitory gene or restoring the function of an essential gene.

Sickle cell disease and muscular dystrophy, two common human genetic disorders, provide instructive examples of diseases that could be treated or cured by genome editing in the foreseeable future. Sickle cell disease results from a single base-pair change in the DNA that in turn generates a defective protein with destructive consequences in red blood cells. DMD belongs to a set of muscle-wasting diseases that result from DNA sequence changes that disrupt the normal production of a protein required for muscle strength and stability. A closer look at each of these diseases illustrates the ways that genome editing could offer therapeutic benefit to patients.

Sickle cell disease occurs in individuals who have two defective copies of the gene that encodes β-globin (*HBB*), the protein required to form oxygen-carrying haemoglobin in adult blood cells. Described originally by Linus Pauling and colleagues[11] and mapped to a genetic locus in the 1950s[12], a single A-to-T mutation results in a glutamate-to-valine substitution in β-globin (Fig. 1). This seemingly small change causes the defective protein to form chain-like polymers of haemoglobin, inducing red blood cells to assume a sickled shape that leads to occluded blood

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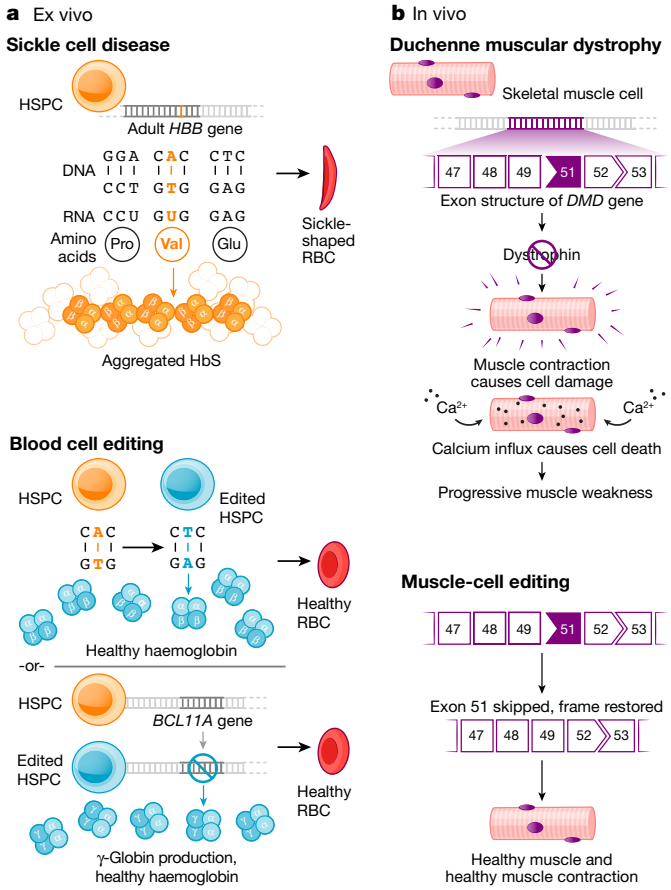

Fig. 1 | Ex vivo and in vivo genome editing to treat human disease. **a**, **b**, Somatic genome-editing treatments may be accomplished in one of two ways: by removing and editing target cells in the laboratory before returning them to the patient (ex vivo, **a**) or by directly delivering CRISPR–Cas editing tools to the affected tissue (in vivo, **b**). **a**, Blood disorders such as sickle cell disease may be treated by editing haematopoietic stem or progenitor cells (HSPCs) ex vivo, creating normal red blood cells (RBCs). **b**, Disorders that affect non-removable tissues, such as DMD, require editing of affected cell types (in this case myogenic cells) in vivo.

vessels, pain and life-threatening organ failure. Although bone-marrow transplantation can cure the disease, it requires the use of cells from an individual whose immune profile matches that of the patient. In principle, sickle cell disease could be cured by removing blood stem cells—that is, haematopoietic progenitor cells—from a patient and using genome editing to either correct the disease-causing mutation in β-globin or activate expression of γ-globin, a fetal form of haemoglobin that could substitute for defective β-globin (Fig. 1). The edited stem cells could then be transplanted back into the patient, in whom the progeny of these edited stem cells would produce healthy red blood cells.

The ability to edit cells extracted from patients with sickle cell disease makes this disease—and other blood disorders—one of the more tractable pathologies that could be treated by genome editing in the near future. Most genetic diseases, however, will require genome editing of cells in the body (in situ) to correct a genetic defect associated with a disease. Muscular dystrophy exemplifies this type of disorder, because it involves the weakening and disruption of skeletal muscles over time[13,14]. The most common type, DMD, affects 1 in 5,000 males at birth, who inherit mutations in the gene that encodes dystrophin (*DMD*), a scaffolding protein that maintains the integrity of striated muscles (Fig. 1). Over time, these patients lose the ability to walk and eventually succumb to respiratory and heart failure, typically dying by the third decade of life. In contrast to therapies that delay disease progression, genome editing offers the possibility of permanent restoration of the missing dystrophin protein. Although more than 3,000 different mutations can cause DMD, most occur at hotspots within *DMD*. Notably, restoration of a small percentage (around 15%) of the normal expression levels of dystrophin can provide a clinical benefit[15].

To treat or cure monogenetic disorders such as sickle cell disease and DMD, it will be important to match the underlying genetic defect with the best genome-editing approach. In each case, this involves multiple considerations, including the type of editing needed, the mode of cell or tissue delivery required and the extent of gene knockout or correction that will provide therapeutic value.

The next section describes current genome-editing technologies that offer the potential of curative human genome editing.

Genome-editing strategies

Engineered DNA-cleaving enzymes, including zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), have demonstrated the potential of therapeutic genome editing. These early technologies enabled the inactivation of the gene encoding the HIV co-receptor CCR5 in somatic cells[16], mitigation of the *HBB* gene mutation in haematopoietic stem cells[17,18] and engineering of immune cells for the treatment of childhood cancer[19]. To realize this potential, the development of CRISPR–Cas9 for genome editing offers a simpler technology that has been adopted widely owing to the ease of programming of its DNA-binding and modifying capabilities. Cas9 is a protein that assembles with a guide RNA—either as separate crRNA and tracrRNA components or a chimeric single-guide RNA (sgRNA)—to create a molecular entity that is capable of binding and cutting DNA[1]. Notably, DNA binding occurs at a 20-base-pair DNA sequence that is complementary to a 20-nucleotide sequence in the guide RNA and that can be readily altered by the researcher[1,20] (Fig. 2). The DNA-recognition site must be adjacent to a short motif (the protospacer adjacent motif or PAM) that acts as a switch, triggering Cas9 to make a double-stranded DNA break within the target sequence[1,20]. In cells of all multicellular organisms, including humans, such double-stranded DNA breaks induce DNA repair by endogenous cellular pathways that can introduce alterations to the DNA sequence, including small sequence changes or genetic insertions[21,22]. Although CRISPR–Cas9-induced genome editing is effective in almost all cell types, controlling the exact editing outcome remains a challenge in the field, as discussed below.

Although the Cas9 of *Streptococcus pyogenes* (SpCas9) is the enzyme that is most commonly used for genome editing and genetic manipulation using CRISPR–Cas, a growing collection of natural and engineered Cas9 homologues and other CRISPR–Cas RNA-guided enzymes is expanding the genome-manipulation toolbox[6,23,24]. It is the intrinsic programmability that is present in this diversity of enzymes that underscores the utility of CRISPR–Cas technology for genome editing and other applications including gene regulation and diagnostics (Fig. 2).

For safe and effective clinical use ex vivo and in vivo, genome editing needs to be accurate, efficient and deliverable to the desired cells or tissues. CRISPR–Cas9-generated DNA cleavage induces genome editing during double-stranded DNA break repair by non-homologous end joining and/or homology-directed repair (Fig. 2). Homology-directed repair, which requires the presence of a DNA template, is—in most cases—used by the cell less frequently than non-homologous end joining. Furthermore, both types of repair can happen in the same cell, creating different alleles of an edited gene. Two concurrent double-stranded DNA breaks can induce chromosomal translocations. For these reasons, an active area of CRISPR–Cas technology development involves controlling DNA repair outcomes to ensure that the desired genetic change is introduced.

Alternatives to DNA-cleavage-induced editing include using CRISPR–Cas9 to directly alter the chemical sequence (base editing)[25,26], to generate RNA templates for gene alteration (prime editing)[27,28] and for transcriptional control (CRISPR interference and

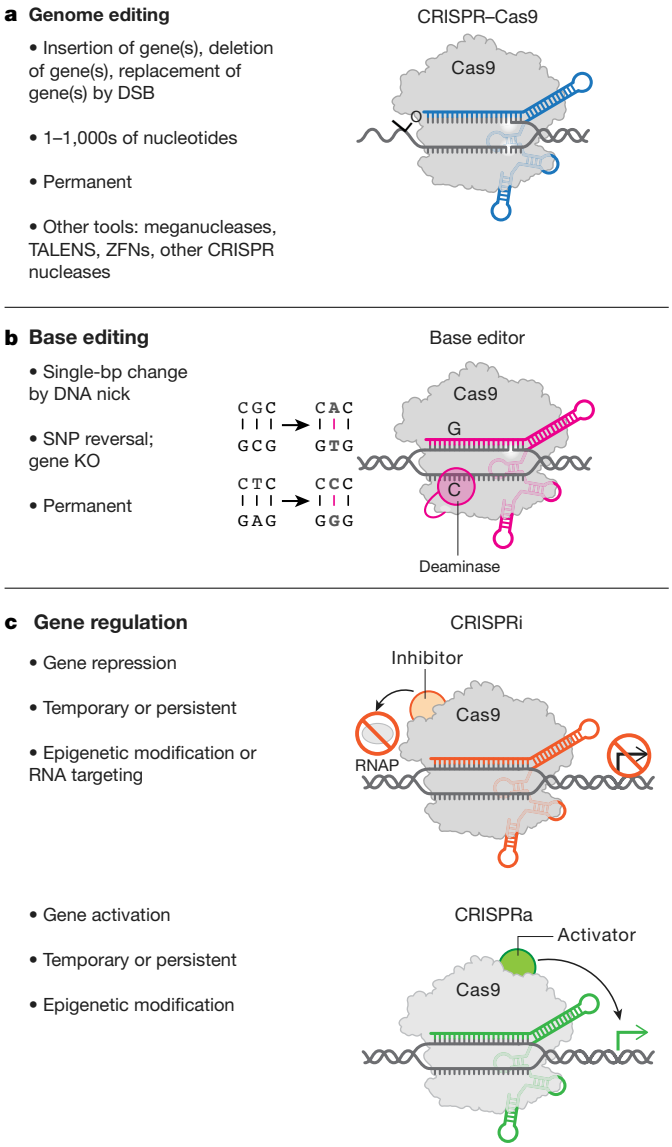

Fig. 2 | The genome editing toolbox. a–**c**, Most well-validated CRISPR-based tools perform one of three functions: genome editing (**a**), base editing (**b**) or gene regulation (**c**). These systems rely on RNA-guided Cas9 or Cas12a to target specific genomic sites. These techniques edit the target site by direct cleavage of one or both nuclease active sites, triggering cellular DNA repair by non-homologous end joining or homology-directed repair, and/or by relying on fused effector proteins. **a**, CRISPR–Cas9 generates a double-stranded break (DSB) at the target site to simulate endogenous DNA repair. These double-stranded breaks are resolved by the endogenous cellular repair machinery, resulting in one of two main outcomes at the cut site: an insertion or deletion, or the insertion of or replacement with donor DNA that is delivered at the same time. **b**, A fused domain replaces a singe base through deamination and DNA replication or repair. This single base change is propagated to the complementary strand of DNA. Changes include C to U (uracil), which is swapped to a T during replication or repair, and A to I (inosine), which is treated as a G. bp, base pair; KO, knockout. **c**, CRISPR-mediated gene repression or interference (CRISPRi) sterically blocks the RNA polymerase and induces heterochromatinization, leading to direct epigenetic modifications such as DNA methylations or RNA targeting by modifying individual bases or RNA cleavage. CRISPR-mediated gene activation (CRISPRa) recruits the transcription machinery to increase expression of the target region and leads to direct epigenetic modifications such as histone acetylation.

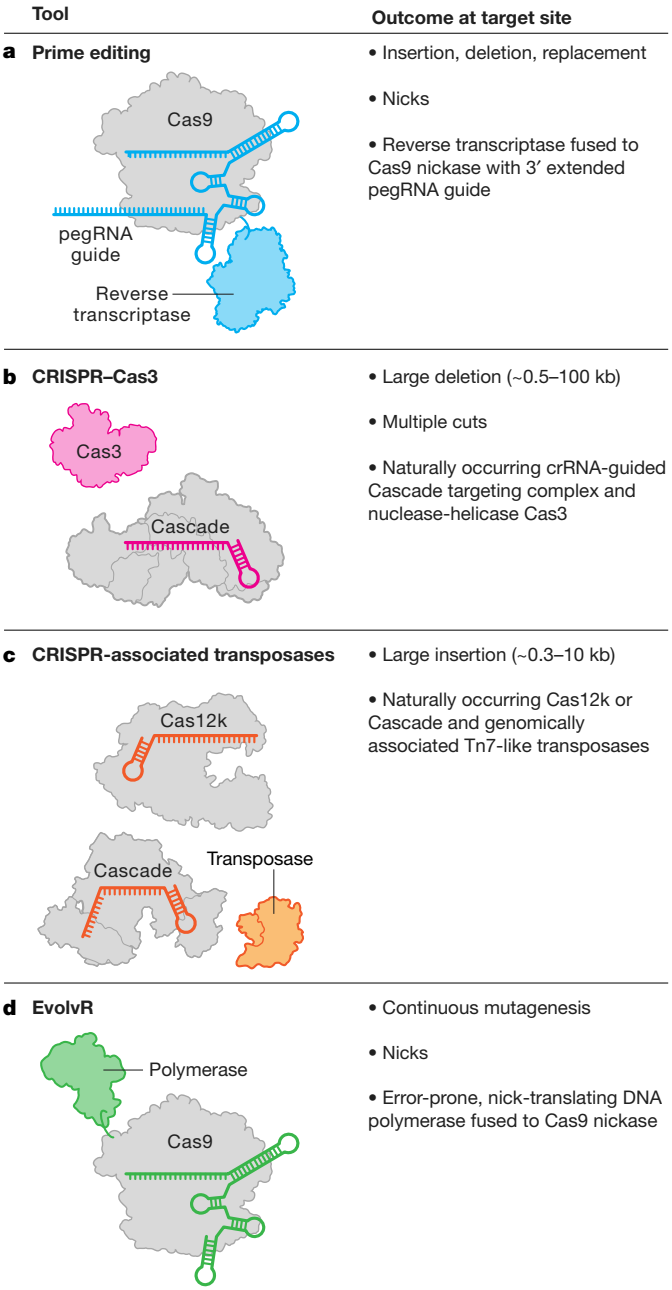

Fig. 3 | Emerging tools. New modifications of the CRISPR–Cas platform are currently underway and, if validated, could provide specific genome modification specialties. **a**, Cas9 binds to and nicks the genomic target, after which the reverse transcriptase copies the sequence of the prime-editing guide RNA (pegRNA) to the target site. **b**, Cascade binds to a genomic target, inducing processive cleavage by Cas3 and generating large deletions. **c**, Cascade or Cas12k binds to the genomic target and directs donor DNA insertion by the Tn7-like transposase. **d**, Cas9 binds to and nicks the genomic target, after which the error-prone polymerase generates diversity in an adjacent window, thus enabling directed evolution.

CRISPR activation)[29,30] (Fig. 3). In addition, it may be possible to control gene outputs through Cas9-mediated epigenetic modification[31,32]. Although these methods have been used in cultured cells, they are not yet ready for clinical use until matters of specificity[33,34] and delivery are addressed.

Two strategies to mitigate or cure sickle cell disease take advantage of demonstrated strategies for site-specific genome editing (Figs. 1, 2). The first involves the restoration of the wild-type *HBB* gene sequence by homology-directed repair[35]. The second approach is to activate expression of γ-globin, the fetal form of haemoglobin that is typically silenced in adult cells, by disrupting γ-globin repressors[36–41] or their

binding sites in the promoter of the γ-globin (*HBG1*/*HBG2*) genes[40,42,43]. These genome-editing strategies require the collection of a patient's haematopoietic stem and progenitor cells, either to correct the mutation in *HBB* or to restart expression of γ-globin, and the subsequent reintroduction of the edited cells into the bone marrow. Major progress in the delivery[44] and handling of haematopoietic stem and progenitor cells has resulted in impressive efficiencies of mutation correction or mitigation[18,45–47] that are expected to be curative.

Such an approach, although it requires a bone-marrow transplantation, would remove the need for a compatible bone-marrow donor and thus provide a path for treating and potentially curing many more people than can currently be treated. As discussed below, improvements in in vivo delivery technology may one day enable treatment without requiring bone-marrow transplantation, which would reduce both expense and patient hardship.

Whereas in vivo editing may resolve some of the issues with ex vivo sickle cell therapies, studies in DMD illustrate that other challenges arise when attempting in situ gene correction. Three reports[48–50] have highlighted both the tremendous potential of genome editing and the considerable challenges that remain before genome editing can be used to treat or cure muscular dystrophy in humans. In the first study, a mouse model of DMD was created using CRISPR–Cas9 to generate a common deletion (ΔEx50) in the *Dmd* gene that also occurs in patients with DMD[48]. The severe muscle dysfunction in the ΔEx50 mice was corrected by systemic delivery of an adeno-associated virus (AAV) that encoded the CRISPR–Cas9 genome-editing components, restoring up to 90% of dystrophin protein expression throughout the skeletal muscles and hearts of ΔEx50 mice. The second study used CRISPR–Cas9-mediated genome editing to remove a mutation in exon 23 in the *mdx* mouse model of DMD, providing partial recovery of functional dystrophin protein in skeletal myofibres and cardiac muscle[25,26,49]. In the third study, dogs with the ΔEx50 mutation, which corresponds to a mutational 'hotspot' in the human *DMD* gene, were treated using CRISPR–Cas9[50]. After virus-mediated systemic delivery in skeletal muscle, dystrophin levels were restored to 3–90% of normal, and the appearance of the muscle tissue in treated dogs was improved. Although promising, these reports, as well as early-stage data from patients treated with in vivo gene editing using ZFNs, highlight the gap between animal studies and applications in humans[51–53] and underscore the need for improved methods for in situ delivery, as discussed in the next section. An early-stage clinical trial in which in vivo CRISPR–Cas9 delivery to the eye is used to treat congenital blindness[54] and a close-to-the-clinic program for liver gene editing[55] will soon provide key first-in-human data to inform the direction of that effort.

Towards tissue-specific delivery

For any of these genome-editing methods to be useful clinically, the CRISPR–Cas enzymes, associated guide RNAs and any DNA repair templates must make their way into the cells that are in need of genetic repair. To produce a functional genome-editing complex, Cas9 and sgRNA can be introduced to cells in target organs in formats that include DNA, mRNA and sgRNA, or protein and sgRNA. All three formats are currently—or will soon be—used in the clinic, using viral vectors, nanoparticles and electroporation of protein–RNA complexes, and each has distinct benefits and limitations (Table 1). The currently favoured form of ex vivo delivery to primary cells is electroporation of Cas9 as a preformed protein–RNA (ribonucleoprotein (RNP)) complex[44,56]. In vivo delivery, which is much more challenging, is currently conducted using viral vectors (typically AAVs) or lipid nanoparticles bearing Cas9 mRNA and an sgRNA. The difficulty of ensuring efficient, targeted delivery into desired cells in the body currently limits the clinical opportunities of in vivo genome editing, although this is an area of increasing research and development.

Viral delivery vehicles, including lentiviruses, adenoviruses and AAVs, offer advantages of efficiency and tissue selectivity (Table 1). AAVs are attractive because of the reduced risk of genomic integration, inherent tissue tropism and clinically manageable immunogenicity. In addition, long-term expression of trans-genes that encode Cas9 and sgRNA from the episomal viral genome could help to boost genome-editing efficiency in patients, such as individuals with DMD as discussed below[57]. Notably, the FDA has approved the use of AAVs for gene-replacement therapy in patients with spinal muscular atrophy and congenital blindness, and clinical trials are in progress[58].

There are, however, considerable challenges to using AAVs for the therapeutic delivery of CRISPR–Cas components. First, the AAV genome can only encode around 4.7 kilobases (kb) of genetic cargo, less than other viral vectors and not much larger than the 4.2-kb length of the gene that encodes *S. pyogenes* Cas9. As a result, for applications that necessitate the insertion of a corrective gene, a second AAV vector that encodes the sgRNA and a template sequence for homology-directed DNA repair must be used, reducing efficiency owing to the need for cells to acquire both AAV vectors at once[59,60]. Smaller genome-editing proteins, such as the Cas9 of *Staphylococcus aureus* or *Campylobacter jejuni* and other newly identified CRISPR–Cas enzymes, may circumvent this issue[23,61–65]. Second, long-term expression of genome-editing molecules may expose patients to undesired off-target editing or immune reactions[66,67]. Third, the production of AAVs at scale and the use of good manufacturing process methods at affordable cost for clinical use remain formidable challenges[68–70].

Nanoparticles offer an alternative to virus-based delivery of Cas9 and sgRNAs and are suitable for delivering genome-editing components in the form of DNA, mRNA or RNPs (Table 1). For example, the delivery of lipid-mediated nanoparticles has been used to transport CRISPR–Cas components in the form of either mRNA and sgRNA or preassembled RNPs into tissues[71–74]. When combined with a highly anionic sgRNA, the cationic Cas9 protein forms a stable RNP complex that has anionic properties suitable for encapsulation by cationic lipid nanoparticles, potentially enabling delivery into cells through endocytosis and macropinocytosis. Cationic lipid-based delivery is a relatively easy, low-cost process for delivering CRISPR components into cells[75]. This approach has been used for one-shot delivery of Cas9 RNPs into mice to achieve therapeutically useful levels of genome editing in the liver[55]. Disadvantages of this approach include marked toxicity of the lipid-mediated nanoparticles[76] and the potentially undesired selectivity of cell-type-specific uptake of the particles.

Inorganic nanoparticles are another type of delivery vehicle with advantages that include tunable size and surface properties. Gold nanoparticles, in particular, are attractive materials for molecular delivery because of the intrinsic affinity of gold for sulfur, enabling functionalized molecules to be coupled to the gold particle surface. Gold nanoparticles were used originally for nucleic acid delivery by conjugating to thiol-linked DNA or RNA[77]. Cas9 protein–sgRNA complexes can be incorporated by assembly with DNA-linked particles[78]. Such assemblies, complexed with polymers capable of disrupting endosomes and including DNA templates for homology-directed repair, were found to promote correction of *Dmd* gene mutations in mice[79]. Ongoing research continues to advance nanoparticle delivery technology, such as for endothelial cells that could enable access to the lungs and other organs[80].

Strategies for non-viral cellular delivery of CRISPR–Cas components include electroporation, which involves pulsing cells with high-voltage currents that create transient nanometre-sized pores in the cell membrane. This process allows negatively charged DNA or mRNA molecules or CRISPR–Cas RNPs to enter the cells. Although this method is a primary method of Cas9–sgRNA delivery to cells ex vivo, electroporation has also been used successfully for Cas9 delivery to animal zygotes[81,82], and to introduce CRISPR–Cas constructs directly into the skeletal muscle in mice, resulting in restoration of *Dmd* gene expression[83]. Electroporation will likely be of limited utility for most in vivo genome-editing applications because of its impracticality.

Table 1 | Methods for delivering genome-editing tools

Property	Nanoparticles	Viruses	RNPs
Features and applications	Cationic lipid polymers can be used to encapsulate molecular cargo, facilitating cellular entry.	AAVs are the most commonly used clinical delivery vehicle for gene therapy.	Purified protein and guide RNA can be electroporated into stem cells extracted from patients to treat blood disorders such as sickle cell disease.
Size	50–500 nm	20 nm	12 nm
Payload	mRNA, DNA, RNP (from most to least commonly used)	DNA	Preformed enzyme complexes
Advantages	- Inexpensive and relatively easy to produce - No genomic integration - Low immunogenicity	- Broad tissue targeting possibilities - Clinically established method - Efficient	- No genomic integration - No long-term expression and fewer off-target effects
Disadvantages	- Limited capacity for tissue targeting	- Limited cargo size - Undesired integration risk - Sustained expression can lead to off-target effects - Immunogenicity - High cost and manufacturing challenges	- Will not enter cells without engineering or additional reagents - Potential immunogenicity in vivo - Unprotected RNPs are at risk of degradation
Targets	Liver	Liver, eyes, brain, lungs and muscle	Oocytes, stem cells and T cells

The three main delivery strategies that could be used for clinical genome-editing applications are nanoparticles, viruses and purified RNPs. The approaches vary in important ways, which generally limit their suitability for editing to specific cell or tissue types.

Another non-viral delivery method is the direct application of pre-assembled CRISPR–Cas RNPs, with or without chemical modifications to assist cell penetration of cultured cells or organs. This delivery mode can reduce possible off-target mutations relative to delivering Cas9-encoding DNA or mRNA due to the short half-life of RNPs[76,84–86]. New strategies for the direct delivery of CRISPR–Cas9 RNP complexes continue to emerge, including those using molecular engineering to enhance the targeting of specific cell types[87] and to increase the efficiency of cell penetration[88].

Delivery remains perhaps the biggest bottleneck to somatic-cell genome editing, a reality that has motivated increasing effort across different disciplines. Emerging strategies that may have substantial impact on the clinical use of genome editing include advances in nanoparticle- and cell-based delivery methods[89] as well as approaches that involve red blood cells[90] and nanowires[91].

Accuracy, precision and safety of genome editing

The clinical utility of genome editing depends fundamentally on accuracy and precision. Accuracy refers to the ratio of on- versus off-target genetic changes, whereas precision relates to the fraction of on-target edits that produce the desired genetic outcome. Inaccurate (off-target) genome editing occurs when CRISPR-induced DNA cleavage and repair happens at genome locations not intended for modification, typically sites that are close in sequence to the intended editing site[92]. Imprecise genome editing results from different modes of DNA repair after on-target DNA cleavage, such as a mixture of non-homologous end-joining and homology-directed recombination events that produce different sequences at the desired editing location in different cells. In addition, large deletions and complex genomic rearrangements have been observed after genome editing in mouse embryonic cells, haematopoietic progenitor cells and human immortalized epithelial cells[93–95]. Although these events occur at low frequency, they could be important in a clinical setting if rare translocations led to cancer[96–98]. Careful testing will be required to detect and monitor both the accuracy and precision of genome editing in clinical settings and ultimately to reduce or eliminate undesired events by controlling target site recognition and DNA repair outcomes. The National Institute of Standards and Technology manages a scientific consortium that aims to measure and standardize such outcomes as genome-editing technology advances[99].

The risks intrinsic to DNA-cleavage-induced genome editing have spurred the development of CRISPR–Cas9-mediated genome regulation or editing methods that do not involve double-stranded DNA cutting. CRISPR interference and CRISPR activation both use catalytically deactivated forms of Cas9 (dCas9) that are fused to transcriptional repressors or activators[29,100]. Similarly, CRISPR–Cas9-mediated epigenetic modification to control gene expression is also under development[101]. An alternative approach is to use CRISPR–Cas9 coupled to DNA-editing enzymes that catalyse targeted A-to-G or C-to-T genomic sequence changes without inducing a break in the DNA, potentially reversing pathogenic single-nucleotide changes or disabling genes through the introduction of a stop codon[25,26]. CRISPR–Cas9 can also be linked to reverse transcriptase and used for targeted template-directed sequence alterations[102]. All of these strategies—although elegant in principle—involve large chimeric proteins that pose additional challenges of delivery into primary cells or animals. The specificity of action, both at the target site and genome-wide, remains an area of active investigation. Issues of delivery, potency and specificity of CRISPR interference, CRISPR activation and CRISPR-mediated base editing and prime editing will need to be thoroughly addressed before they are ready for clinical use.

Other factors that affect clinical applications of genome editing include the immunogenicity of bacterially derived editing proteins, the potential for pre-existing antibodies against CRISPR components to cause inflammation and the unknown long-term safety and stability of genome-editing outcomes. Immunogenicity of CRISPR–Cas proteins could be managed by high-efficiency one-time editing treatments and by using different editing enzymes. Pre-existing Cas9 antibodies and reactive T cells have been detected in humans exposed to pathogenic bacteria that have CRISPR systems, although it is unknown whether these are present at sufficiently high concentrations to trigger an immune response to the genome-editing enzymes[66,103]. Notably, genome-editing therapies that involve ex vivo editing, such as for sickle cell disease, are not as affected by either immunogenicity or pre-existing CRISPR–Cas antibodies, as the natural decay of residual Cas9 protein in the ex vivo edited cells minimizes Cas9 exposure. The potential for inadvertent selection of genome-edited cells with undesired genetic changes came to light with the observation that selection for inactivation of the p53 pathway, which is associated with rapid cell growth and cancer, can occur during laboratory experiments on cells that are not used clinically[104,105]. Subsequent experiments showed that p53 inactivation can be controlled or avoided through protocol optimization[47,106]. As for the long-term safety and efficacy of genome-edited cells in vivo, much remains to be determined. However, the recent report

of a single HIV-positive patient who received CRISPR–Cas9-edited haematopoietic progenitor cells showed that although the number of edited cells was too low to mitigate HIV infection, no adverse outcome was detected more than 19 months after transplantation of the edited cells[107]. Together, these findings suggest that there are, at present, no known insurmountable hurdles to the eventual development of safe and effective clinical applications of genome editing in humans.

Therapeutic genome editing

The clinical potential of genome editing exemplified by applications in sickle cell disease, muscular dystrophy and other monogenetic disorders could be stymied by extreme pricing of such next-generation therapeutics. Although CRISPR technology itself is a democratizing tool for scientists, extension of its broad utility in biomedicine requires addressing the costs of development, personalization for individual patients and the intrinsic difference between a chronic disease treatment versus a one-and-done cure[102].

Current clinical trials using the CRISPR platform aim to improve chimeric antigen receptor (CAR) T cell effectiveness, treat sickle cell disease and other inherited blood disorders, and stop or reverse eye disease[108]. In addition, clinical trials to use genome editing for degenerative diseases including for patients with muscular dystrophy are on the horizon. For sickle cell disease, the uniform nature of the underlying genetic defect lends itself to correction by a standardized CRISPR modality that could be used in many if not most patients. This simplifies clinical testing but also makes the need to address patient cost and access more acute, given that the approximately 100,000 US patients and millions of individuals in African and Asian countries will be candidates for treatment.

For muscular dystrophy, the genetic diversity among patients lends itself to personalization, which is an inherent strength of the CRISPR genome-editing platform; however, it also complicates clinical testing strategies. In addition, progressive diseases such as muscular dystrophy require early treatment to be most effective, raising questions about coupling diagnosis and treatment. Beyond these examples, many rare genetic disorders will be treatable—in principle—if a streamlined strategy for CRISPR therapeutic development can be implemented[102]. With its potential to address unmet medical needs, the clinical use of genome editing will ideally spur changes to regulatory guidelines and cost reimbursement structures that will benefit the field more broadly as these therapies continue to advance.

Notably, all of the genome-editing therapeutics under development aim to treat patients through somatic cell modification. These treatments are designed to affect only the individual who receives the treatment, reflecting the traditional approach to disease mitigation. However, genome editing offers the potential to correct disease-causing mutations in the germline, which would introduce genetic changes that would be passed on to future generations. The scientific and societal challenges associated with human germline editing are distinct from somatic cell editing and are discussed in the next section.

Heritable genome editing

Human germline genome editing can introduce heritable genetic changes in eggs, sperm or embryos. Germline genome editing is already in widespread use in animals and plants, and has been used in human embryos for research purposes. A report of alleged use of human embryo editing that resulted in the birth of twin baby girls with edited genomes has focused global attention on an application of genome editing that must be rigorously regulated, as underscored by international scientific organizations.

Human germline editing differs from somatic cell editing because it results in genetic changes that are heritable if the edited cells are used to initiate a pregnancy (Fig. 4). Germline editing has been used for years in animals, including mice, rats, monkeys and many others,

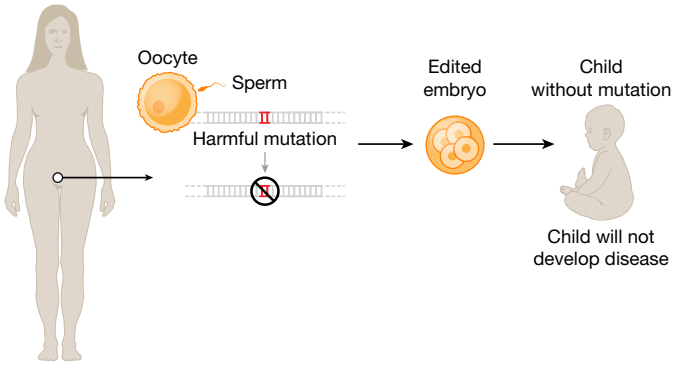

Fig. 4 | Editing the human germline. Genomic changes made during or after embryogenesis may be found in some (mosaic) or all of the cells of the child, including the germline. In contrast to somatic editing (Fig. 1), germline-edited humans can pass these edits down to subsequent generations. In the first human germline-editing experiment in embryos carried to term, the stated goal was to confer HIV resistance, making this example relevant to the real world and highlighting the potential problematic nature of this technique.

and experiments show that it can also be done in both nonviable and viable human embryos[109–112]. Although none of the published work involves implantation of the edited embryos to initiate a pregnancy, such clinical work was reported at a conference on human genome editing in November 2018, leading to international condemnation in light of clear violations of ethical and scientific guidelines.

This work and the accompanying discussion around human germline editing have raised important questions that affect the future direction of the science as well as the societal and ethical issues that accompany any such applications. First, research using CRISPR–Cas9 in human embryos has challenged our current understanding of DNA repair mechanisms and the developmental pathways that occur in these cells. A report of inaccurate CRISPR–Cas9-based genome editing in non-viable human embryos[109] was not substantiated by later publications, but the mechanism by which double-stranded DNA breaks are repaired in early human embryos remains under debate. Some results were interpreted to indicate repair of a CRISPR–Cas9-targeted gene allele by homology-directed repair with the other allele of the cell as the donor template[113]. Other scientists argued that such repair would be impossible given the apparent physical separation of sister chromatids early in embryogenesis, and suggested that the data could also be consistent with large deletions in the embryo genomes[93,114]. Resolving this fundamental question will require further experiments. Human embryo editing has also begun to reveal differences in the genetics of early development between mice and humans[110], underscoring the potential value of research that will be enabled by precision genome modification.

A second question raised by applications of genome editing in human embryos concerns the appropriate professional and societal response. Organizations including the National Academy of Sciences, the National Academy of Medicine, the Royal Society and their equivalents in other countries have sponsored meetings and reports, as have professional societies including the American Society of Human Genetics[115], UK Association of Genetic Nurses and Counsellors, Canadian Association of Genetic Counsellors, International Genetic Epidemiology Society, US National Society of Genetic Counselors, American Society for Reproductive Medicine, Asia Pacific Society of Human Genetics, British Society for Genetic Medicine, Human Genetics Society of Australasia, Professional Society of Genetic Counselors in Asia, and Southern African Society for Human Genetics. These groups agree on a number of key points. First, at this time, given the nature and number of unanswered scientific, ethical and policy questions, it is inappropriate to perform germline genome editing that culminates in human pregnancy. Second, in vitro germline genome editing on human

embryos and gametes should be allowed, with appropriate oversight and consent from donors, to facilitate research on the possible future clinical applications of gene editing, and there should be no prohibition on public funding of this research. Third, future clinical applications of human germline genome editing should not proceed unless, at a minimum, there is (a) a compelling medical rationale, (b) an evidence base that supports its clinical use, (c) an ethical justification and (d) a transparent public process to solicit and incorporate stakeholder input.

The third question raised by applications of CRISPR–Cas9 in human embryos is how to move the technology forward while ensuring responsible use. At the time of writing, international commissions convened by the World Health Organization (WHO) and by the US National Academy of Sciences and National Academy of Medicine, together with the Royal Society, are drafting detailed requirements for any potential future clinical use. Medical needs must be defined so that risks versus possible benefits can be evaluated. Most importantly, procedures by which patients could be informed about the technology, its risks and a process for monitoring health outcomes must be determined.

Outlook

Therapeutic genome editing will be realized, at least for some diseases, over the next 5–10 years. This profound opportunity to change healthcare for many people requires scientists, clinicians and bioethicists to work with healthcare economists and regulators to ensure safe, effective and affordable outcomes. The potential impact on patients is too important to wait.

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Competing interests J.A.D. is a co-founder of Caribou Biosciences, Editas Medicine, Intellia Therapeutics, Scribe Therapeutics and Mammoth Biosciences; a scientific adviser to Caribou Biosciences, Intellia Therapeutics, Scribe Therapeutics, Synthego, Inari and eFFECTOR Therapeutics; and a director of Johnson & Johnson. The Regents of the University of California have patents issued and pending for CRISPR-related technologies on which J.A.D. is an inventor.

Additional information

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