REVIEW ARTICLE

FRONTIERS IN MEDICINE

A New Class of Medicines through DNA Editing

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GENOME EDITING IS A SET OF METHODS USED TO CHANGE THE DNA OF A cell with single base-pair precision. It is a specific form of gene therapy, and the engineering of cells through genome editing has the potential to create a new class of medicines for the treatment of both genetic and nongenetic diseases. Genome editing has entered clinical trials: applications include the correction of variants that cause monogenic diseases, the enhancement of chimeric antigen receptor (CAR) T-cell therapy, and cell-based regenerative medicine. Here I describe the development of genome editing and discuss the ways in which efficacy, specificity, delivery, and safety are integral to this process (see interactive graphic, available at NEJM.org).

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EARLY DEVELOPMENT OF GENOME EDITING

Until 1994, the efficiency of genome editing in a mammalian cell was 10^{-6} (1 cell in 1 million would have the desired gene-targeting event).[1] In 1994, Jasin and colleagues discovered that the creation of a break in a DNA double strand in a target gene could stimulate gene targeting by a factor of more than 1000 in somatic cells when a "donor" template strand of DNA was provided at the same time that the break was created.[2-5] With optimization, this system could be used to correct a reporter gene in up to 5% of cells.[4] (A reporter gene delivers a signal on successful DNA editing.) In addition to showing that new sequences could be inserted at the site of the break through homologous recombination, this discovery also indicated that new mutations could be created at the site of the break through a process called nonhomologous end-joining (NHEJ).[6-8] The discovery that a specific double-strand break in DNA could induce repair is the foundational principle of the field of genome editing. (See Fig. 1 for a timeline of discoveries.)

A limitation of these early studies was their use of a specific homing endonuclease, an enzyme that recognizes and cuts a specific DNA sequence (a recognition site). This approach could not be applied to human cells because the recognition site does not occur in endogenous genes. The problem was solved by engineering nucleases that recognize target sites in endogenous genes and stimulate genome editing at those sites.[4,7-10] The first such nucleases were zinc-finger nucleases, in which a DNA-binding protein with a specific recognition sequence was fused to a nonspecific nuclease domain.[11] A wide variety of nucleases are now used in addition to zinc-finger nucleases, including homing endonucleases and transcription activator–like effector nucleases (Fig. 2).[7,12] Each creates a site-specific, double-strand break in the genome of the cell that activates repair through NHEJ or homology-directed repair (HDR). Nonnuclease-based systems of genome editing[13-16] are in earlier stages of development.

An illustrated glossary and an interactive graphic on genome editing are available at NEJM.org

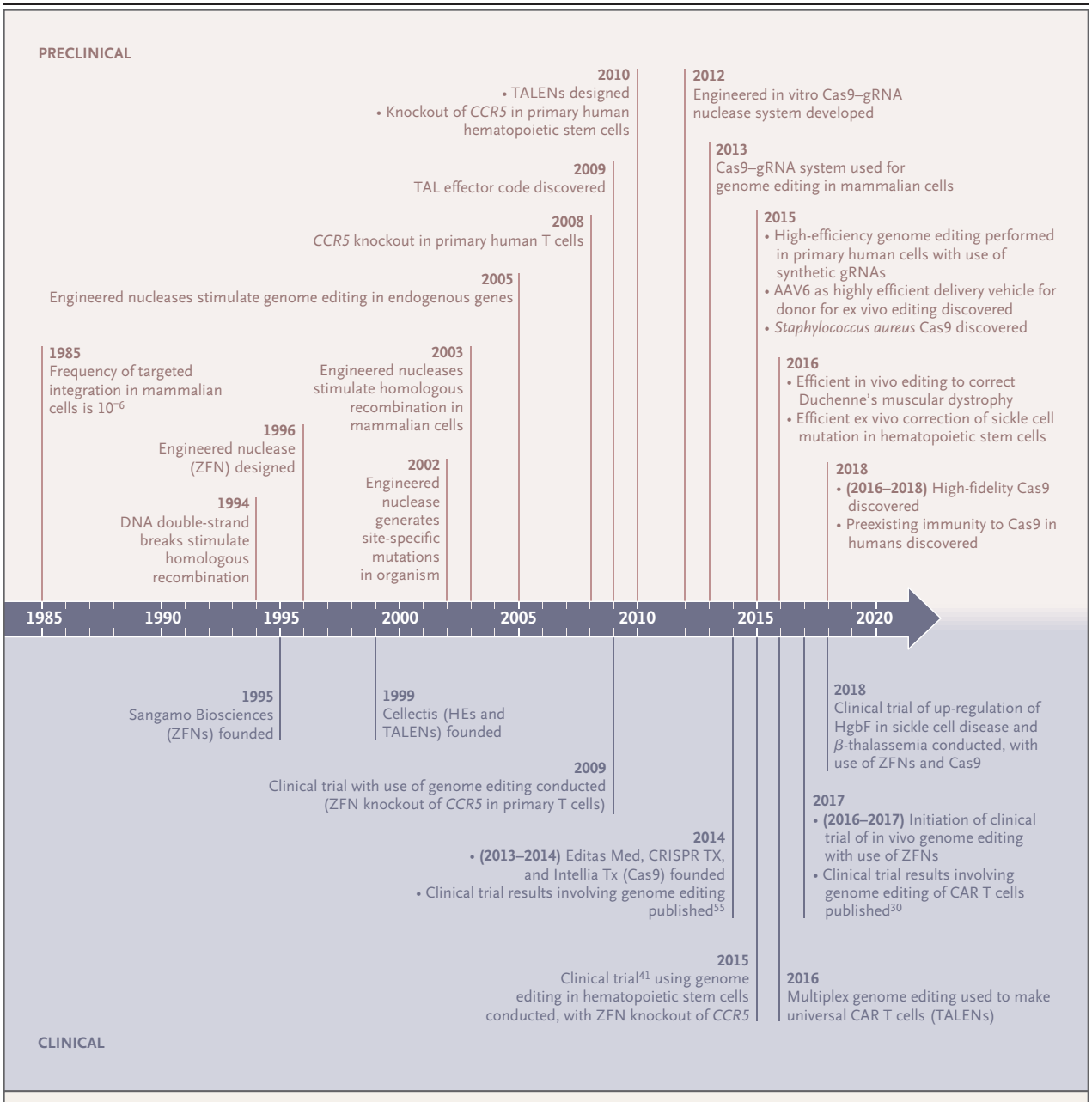


Figure 1. Timeline of Developments in Genome Editing.

AAV denotes adeno-associated virus, CAR chimeric antigen receptor, Cas9 a clustered regularly interspaced short palindromic repeats (CRISPR)–associated protein, CCR chemokine coreceptor, gRNA guide RNA, HE homing endonuclease, TAL transcription activator–like, TALEN transcription activator–like effector nuclease, and ZFN zinc-finger nuclease.

CRISPR–CAS9 NUCLEASE

The nuclease platform known as CRISPR–Cas9 (clustered regularly interspaced short palindromic repeats associated with Cas9 endonuclease) was developed from a bacterial adaptive immune system.[17-20] However, because the only two components of the bacterial system used in genome editing are the Cas9 nuclease and the guide RNA (gRNA), the method is more accurately described

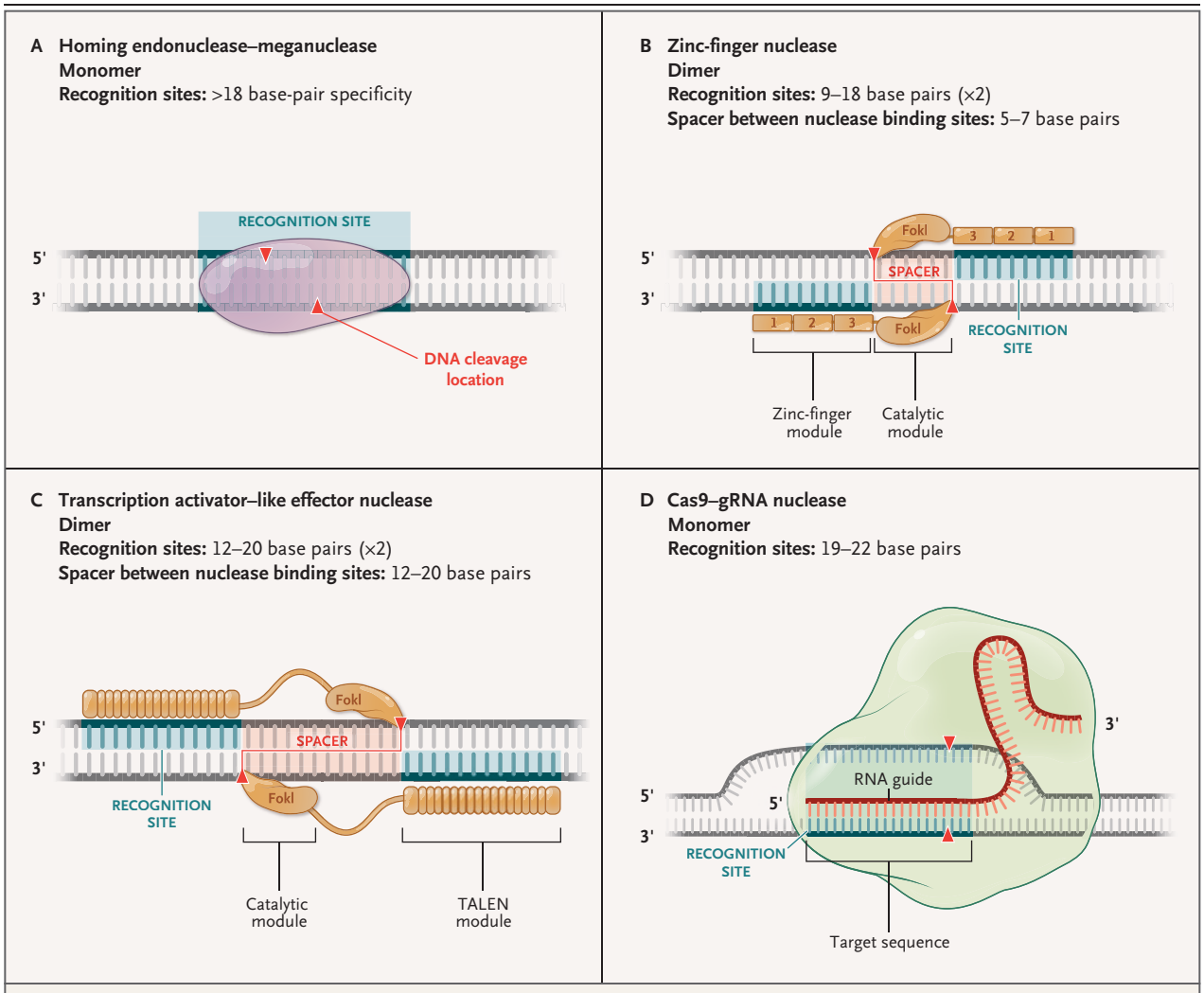


Figure 2. Nuclease Platforms for Genome Editing.

Four major nuclease platforms are used in genome editing: homing endonucleases–meganucleases (Panel A), zinc-finger nucleases (ZFNs) (Panel B), transcription activator–like effector nucleases (TALENs) (Panel C), and the Cas9–gRNA nucleases (Panel D). Shown are the basic structures and key characteristics of each platform in terms of length of recognition site and the action of the nuclease — that is, whether it acts as a dimer or monomer when cutting a DNA strand. The meganuclease, ZFN, and TALEN platforms all derive specificity through protein–DNA binding, whereas the Cas9–gRNA platform derives specificity through Watson–Crick RNA–DNA base pairing.

as the Cas9–gRNA system. In genome editing, the Cas9 nuclease cleaves DNA after an induced conformational change subsequent to the binding of gRNA to the DNA target site.

The most commonly used Cas9 enzyme is from *Streptococcus pyogenes*. The gRNA molecule can be tailored to optimize hybridization with a particular DNA target site and thereby guide the Cas9–gRNA complex to the site of the desired break (Fig. 2).[19,21] In contrast with other genome-editing nuclease systems, the "guidance" of Cas9–gRNA to its target site is governed by Watson–Crick base-pairing, an ease-of-design feature.

DNA EDITING THROUGH NHEJ

NHEJ is a form of double-stranded break repair that does not require a "repair" template.[21,22] Instead, the ends of the broken DNA are held in

close proximity, processed, and then joined. NHEJ-mediated editing is normally used in all cells to repair spontaneous breaks. It is generally accurate (at a rate of at ≥70%)[23,24] but can create errors. NHEJ is the process naturally used by cells of the immune system to create genetic diversity in genes encoding immunoglobulins and T-cell receptors (TCRs).

In the process of ligating the two ends together, the NHEJ machinery may create a small insertion or deletion (an "indel") at the site of the break. Extraneous pieces of DNA in the vicinity of the break can be inserted, a phenomenon that is exploited in certain forms of genome editing.[25,26] It is possible to engineer the integration of a DNA cassette directly into the break through ligation of each end of the DNA fragment to either side of the break, but the frequency of targeted integration with the use of this strategy is low.[27-29] If two simultaneous breaks are created and the breaks are in close proximity on the same chromosome, high frequencies of defined deletions will result. Translocations (which may be pathogenic) are created if the breaks are on different chromosomes (which occurs at a rate of 0.2 to 0.4% in primary human hematopoietic stem and progenitor cells [HSPCs] and of 2 to 4% in primary human T cells).[23,30,31] NHEJ-mediated genome editing has been used in a variety of strategies with potential for therapeutic application (Fig. 3A).

Figure 3 (facing page). Genome Editing through Nonhomologous, End-Joining (NHEJ) Homology-Directed Repair.

Panel A shows genome-editing through NHEJ. Genome editing can use the NHEJ mechanism of DNA double-strand break repair in several ways. Depicted are three major approaches to NHEJ-mediated genome editing and examples of how each approach is being developed to treat specific diseases. Indel refers to a nontemplated creation of small insertions or deletions at the site of the break. Panel B shows genome editing through homology-directed repair. In genome editing, either homologous recombination or single-stranded template repair can be used to create nucleotide-specific changes in the genome. Shown are schematic representations of different applications of both approaches to creating changes in the genome that have single-nucleotide precision. *ALB* denotes the gene encoding albumin, HSC hematopoetic stem cell, HIV human immunodeficiency virus, indel insertion or deletion, and SCIDX1 X-linked severe combined immunodeficiency.

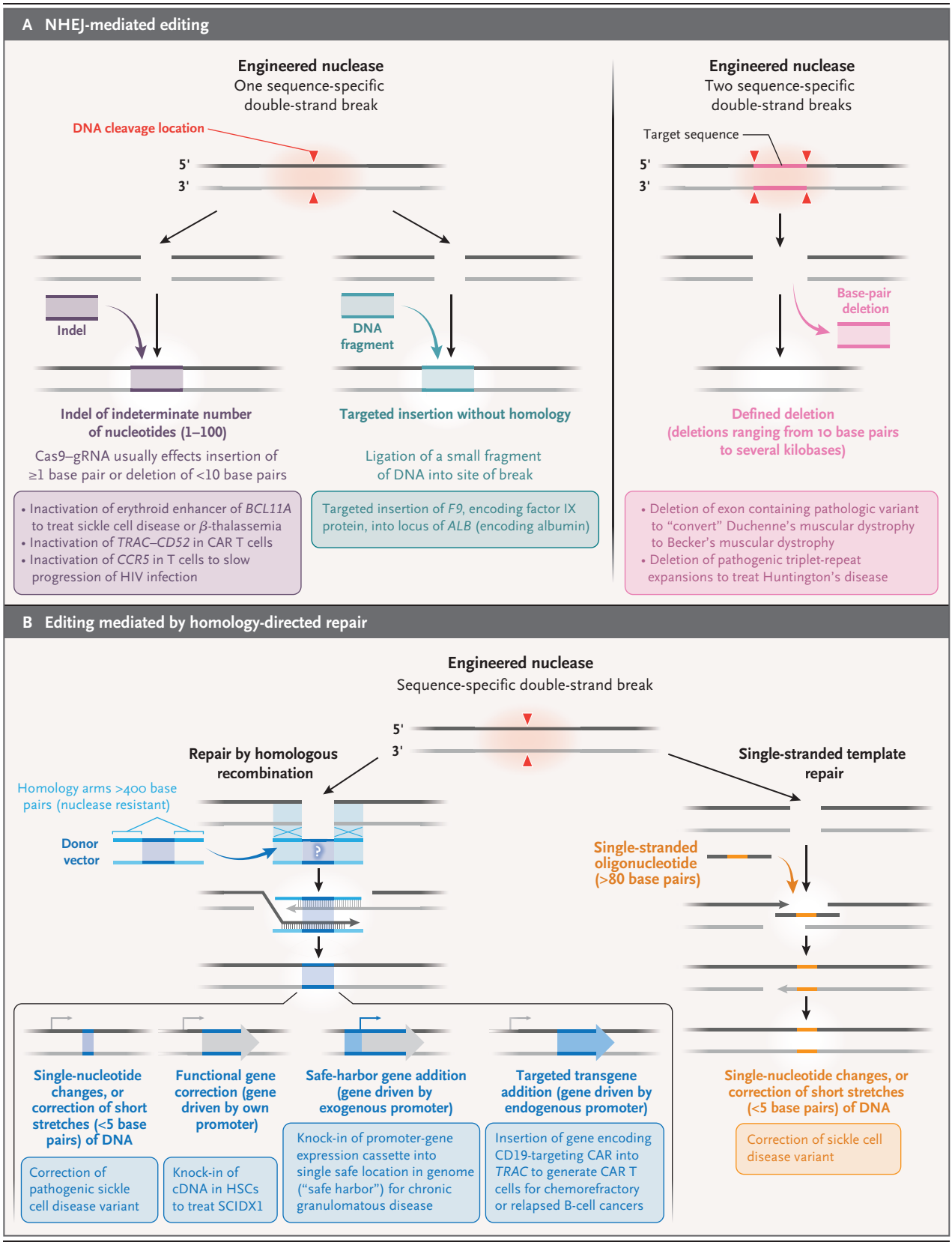
DNA EDITING THROUGH HDR

There are two mechanistically distinct types of HDR.[7,12,32,33] In HDR, a donor DNA template is introduced into the cell, allowing the cell to repair a break with the donor DNA used as a template. A classic gene-targeting donor template has homology arms (each exceeding 400 bases) that flank the genetic change. In homologous recombination, the cell uses its molecular recombination machinery to synthesize new DNA that is complementary to the template, and the new DNA is then used to fix the break through recombination. This form of genome editing is used naturally in processes such as meiotic recombination. Edits of various sizes can be made, from single-nucleotide changes to the insertion of large, multigene cassettes (Fig. 3B). Donor templates can be delivered through a variety of means, including viral vectors and naked DNA molecules.

Fig. 3B shows some of the different approaches to HDR genome editing that are being applied to the treatment of human disease. These include the direct reversion of a disease-causing variant in a gene[34-36]; the insertion of a complementary DNA (cDNA) cassette containing a specific gene into the endogenous locus of that gene such that it is regulated by its own natural regulatory elements[37,38]; the insertion of a cDNA cassette into a different locus such that it will be expressed according to the regulatory elements of the gene at that locus[39]; and the insertion of a transgene cassette into a "safe harbor" to avoid creating unintended insertional mutations caused by semi-random integration with viral vectors (particularly integrations with highly expressed genes) and to achieve more homogeneous expression of the transgene.[40]

THE IMPORTANCE OF DELIVERY

To achieve highly efficient editing, sufficient quantities of a highly active nuclease with good specificity must be delivered into the nucleus of a cell without activating a toxic cellular response. In cancer-cell lines, sufficient nuclease expression can often be generated by transfecting cDNA (encoding the nuclease) into the cells. In primary human cells, however, which have an intact antiviral, cytoplasmic DNA-sensing mech-



anism, the nuclease must be delivered as a messenger RNA (mRNA) molecule (which the cell then translates) or as a ribonucleoprotein complex, such as for Cas9–gRNA.[23,41] Electroporation is an effective and relatively nontoxic method of delivering these molecules ex vivo.

There are other aspects of delivery to consider. For example, although mRNA is better than plasmid DNA in delivering the nuclease to primary human cells, mRNA can induce an antiviral type I interferon response.[42] Moreover, prolonged expression of a nuclease or expression of a nuclease with low specificity can result in sustained activation of the p53 pathway, thereby triggering cell-cycle arrest and apoptosis.[43]

High frequencies of HDR-mediated editing can be achieved by delivering sufficient amounts of template DNA to cells without activating a toxic cellular response (e.g., the type I interferon response). Recombinant adeno-associated viral vectors, which have evolved to avoid cellular detection while delivering single-stranded DNA cargos to the nucleus, are efficient in delivering classic gene-targeting donor templates to cells.[44-47]

Some approaches that enhance HDR-mediated editing in cells involve the use of small molecules to target specific pathways, but the effects of such interventions have been modest and inconsistent; the greatest effects have been realized when the efficiency has not been optimized.[48-51] Moreover, caution is warranted: some of these interventions perturb the ways in which a cell normally repairs or responds to a double-strand break and may therefore compromise the repair of the 20 to 40 double-strand breaks that occur spontaneously in every cell as it progresses through its cycle.

EX VIVO GENOME EDITING TO GENERATE CELLS AS DRUGS

Of all the approaches to genome editing, the most developed is ex vivo genome editing, in which cells are engineered outside of the body and then returned to the patient. Indeed, ex vivo engineering of cells with viral vectors (in standard gene therapy) provides commercially available products that are being used to treat a genetic immunodeficiency and cancer.

Ex vivo NHEJ-mediated genome editing has been tested in clinical trials (Fig. 1 and Table 1), including the treatment of persons infected with the human immunodeficiency virus (HIV). T cells from these patients were obtained, edited (to knock out *CCR5*, which encodes a coreceptor for HIV), and then reinfused in the patients. This approach was found to be safe and was associated with a reduction — albeit a modest one — in the rate of increase of HIV-infected T cells.[55]

In addition, genome editing has been used to make CAR T cells "universal" through the simultaneous disruption of the genes encoding TCRα and CD52, conferring resistance to alemtuzumab, the drug used for lymphodepletion.[30] These CAR T cells are being tested in the treatment of resistant leukemia. Two patients treated on an emergency basis were reported to have remission within 28 days, although graft-versus-host disease developed as a result of residual TCR-positive cells.[30]

These trials use zinc-finger nuclease and transcription activator–like effector nuclease platforms (Fig. 2). Within the next several years, however, multiple clinical trials involving ex vivo modification of HSCs and T cells that use the Cas9–gRNA system will be initiated in the United States and Europe. The trials include the use of NHEJ-mediated knockout strategies to generate more potent CAR T cells to treat cancer.[58] They also include the knockout of the erythroid-specific enhancer of *BCL11A* to up-regulate gamma globin within the erythroid lineage of autologous HSCs[59] as a potential therapy for both sickle cell disease and β-thalassemia.

Human HDR-based genome-editing strategies are also likely to enter clinical trials in the next year. These include direct correction of the variant that causes sickle cell disease in patient-derived HSCs and the generation of more potent CAR T cells.[34,39] Preclinical studies augur well for ex vivo, autologous, cell-based therapies involving genome editing for diseases such as chronic granulomatous disease,[36,46] X-linked severe combined immunodeficiency,[37] X-linked hyper-IgM syndrome,[38] and HIV infection.[44,60]

The overall efficiencies of genome editing of cells ex vivo are remarkably high. It is now routine to generate NHEJ-mediated indels with efficiencies exceeding 80%, large deletions with efficiencies exceeding 50%, and changes effected through HDR at frequencies between 30% and 70% in HSCs and primary human T cells.

GENOME EDITING TO MODIFY CELLS IN SITU

There are many diseases for which ex vivo editing of cells would not provide a clinical benefit. The fact that there is no reliable method for transplanting cells into the liver or brain, for example, represents an obvious barrier. In vivo editing, whereby cells are edited in their natural setting (by delivering the editing apparatus into patients), is a possible solution, and this technique has been applied (with the use of both NHEJ- and HDR-mediated approaches) in preclinical models. Examples include the insertion of a cassette containing a therapeutic protein into a noncoding region of the gene encoding albumin,[52] the creation of indels in *PCSK9* in liver cells to reduce cholesterol levels,[61] the removal of a disease-causing exon in *DMD* to convert severe Duchenne's muscular dystrophy into the milder Becker's muscular dystrophy,[62-64] and the correction of disease-causing variants in mouse models of metabolic diseases.[65,66]

The human immune system (adaptive and innate) has proved to be a consistent barrier to the successful use of genetic engineering in vivo. Transgene immunogenicity is a challenge to standard in vivo gene therapy and to genome-editing strategies. In the context of genome editing, the immune system may also be a barrier to the editing machinery itself. All the major nuclease platforms contain foreign proteins. Prolonged expression of the nuclease is therefore likely to invoke an adaptive immune response, which could eliminate the nuclease-expressing cell, resulting in a lack of efficacy and the generation of toxic effects. In addition, the first dose may vaccinate the patient against subsequent doses.[61] The Cas9 nuclease used in the Cas9–gRNA system is from one of two bacterial species, *S. pyogenes* and *Staphylococcus aureus.* Since each universally infects humans, a large proportion of adults has preexisting immunity to Cas9.[67,68]

SAFETY OF GENOME EDITING

Nuclease-mediated genome editing initiates double-strand breaks, a source of genomic instability that might lead to cancer-causing mutations. Consequently, considerable effort has been focused on understanding and minimizing (through engineering) the creation of off-target double-strand breaks.

Decreasing the duration of nuclease expression — for example, by delivering Cas9–gRNA as a ribonucleoprotein complex — can result in exponential improvements in specificity.[23] This strategy is effective because genome editing is a "hit-and-run" process that does not require sustained nuclease expression. Changing the binding and catalytic activity of the nuclease can similarly result in improved specificity.[7,12,69-71] Changing one component, however, can limit the flexibility in changing another. For example, some Cas9 variants with higher specificity had suboptimal target activity when delivered as a ribonucleoprotein complex.[35] A relatively new Cas9 high-fidelity variant, when delivered as a complex with gRNA, combines high on-target activity with improved specificity.[72]

A challenge in assessing the safety of genome editing is that there are no validated preclinical assays for this new type of medicine. There are different approaches (e.g., bioinformatic, cell capture, and in vitro) that can be used to identify sites that may harbor off-target indels, but no one approach has been established as the most effective, and each has its own intrinsic biases.[12,69]

Although off-target indels can be detected to a certain level of sensitivity, there are no data to provide guidance as to what is a safe level of off-target indels for either ex vivo or in vivo uses of genome editing. It is likely that any engineered nuclease modifying a large population of cells will facilitate translocations between the on-target break and spontaneous, random breaks that continuously arise elsewhere in the genome. Current assays are not sensitive enough to measure the frequency of such events, and they have not been designed to measure the functional consequences of such events. The wisdom of growing a large population of cells from a single clone is uncertain, given that the population could become dominated by a cell with a spontaneous mutation in a tumor-suppressor gene or by an oncogene that is selected for expression during the expansion process. Further complicating the assessment of specificity is the fact that every person has a different genome, with millions of small differences at baseline, which makes it challenging to evaluate the conse-

Table 1. Clinical Trials in Genome Editing, 2009–2019.*

Sponsor	NCT No. (Year, Location)	Disease	Gene Target (Nuclease)	Ex Vivo vs. In Vivo (System)	Delivery Mechanism	Publication
Sangamo Therapeutics	02695160, 02702115, 03041324 (2016–2017, U.S.)	Hemophilia B, mucopolysaccharidosis types I and II	*ALB* (ZFN)	In vivo (liver)	Adeno-associated virus	Sharma et al., 2015[52]‡
Bioverativ	03653247 (2018, U.S.)	Sickle cell disease	*BCL11A* (ZFN)†	Ex vivo (HSPC)	mRNA electroporation	
Sangamo Therapeutics	03432364 (2018, U.S.)	β-thalassemia	*BCL11A* (ZFN)†	Ex vivo (HSPC)	mRNA electroporation	
City of Hope	02500849 (2015, U.S.)	HIV infection	*CCR5* (ZFN)	Ex vivo (HSPC)	mRNA electroporation	Digiusto et al., 2016,[41]‡ Holt et al., 2010[53]‡
University of Pennsylvania	00842634 (2009, U.S.)	HIV infection	*CCR5* (ZFN)	Ex vivo (T cells recognizing NY–ESO antigen)	mRNA electroporation	Perez et al., 2008[54]‡; Tebas et al., 2014[55]§
Sangamo Therapeutics	01252641, 01044654 (2010, U.S.)	HIV infection	*CCR5* (ZFN)	Ex vivo (T cells)	mRNA electroporation	Perez et al., 2008[54]‡
Institut de Recherches Internationales Servier	02808442 (children), 02746952 (adults) (2016, Europe)	Acute lymphoblastic leukemia	*TCR, CD52* (TALEN)	Ex vivo (CD19 CAR T cell)	mRNA electroporation	Poirot et al., 2015[56]‡; Qasim et al., 2017[30]§
Cellectis	03203369 (2017, U.S.)	Blastic plasmacytoid dendritic cell neoplasm	*TCR, CD52* (TALEN)	Ex vivo (CD123 CAR T cell)	mRNA	
Cellectis	03190278 (2017, U.S.)	Acute myeloid leukemia	*TCR, CD52* (TALEN)	Ex vivo (CD123 CAR T cell)	mRNA	
First Affiliated Hospital, Sun Yat-Sen University	03057912 (2017, China)	HPV infection	*E6, E7* (TALEN or Cas9 and gRNA)	In vivo	Plasmid and polymer gel	
Huazhong University of Science and Technology	03226470 (2017, China)	HPV infection	*E6, E7* (TALEN)	In vivo	Plasmid and polymer gel	Hu et al., 2015[57]‡
Chinese PLA General Hospital	03545815 (2018, China)	Solid tumors	*TCR, PD-1* (Cas9 and gRNA)	Ex vivo (CAR T cell–mesothelin)	Not disclosed	

Affiliated Hospital to Academy of Military Medical Sciences	03164135 (2017, China)	HIV infection	*CCR5* (Cas9 and gRNA)	Ex vivo (HSPC)	Not disclosed
Vertex Pharmaceuticals	03655678 (2018, Europe)	β-thalassemia	*BCL11A* (Cas9 and gRNA)	Ex vivo (HSPC)	Ribonucleoprotein electroporation
Vertex Pharmaceuticals	03745287 (2018, U.S.)	Sickle cell disease	*BCL11A* (Cas9 and gRNA)	Ex vivo (HSPC)	Ribonucleoprotein electroporation
University of Pennsylvania	03399448 (2018, U.S.)	Multiple myeloma	*TCRα, TCRβ, PD-1* (Cas9 and gRNA)	Ex vivo (CAR T cell–NY-ESO)	mRNA electroporation
Chinese PLA General Hospital	03398967 (2018, China)	Acute lymphoblastic leukemia, lymphoma	*TCR*	Ex vivo (CD19, CD20, CD22 CAR T cell)	Not disclosed
Chinese PLA General Hospital	03166878 (2017, China)	Acute lymphoblastic leukemia, lymphoma	*TCR, B2M*	Ex vivo (CD19 CAR T cell)	Not disclosed
Shanghai Bioray Laboratory	03229876 (2017, China)	Acute lymphoblastic leukemia	*TCR, HLA-1* (Cas9 and gRNA)	Ex vivo (CD19 CAR T cell)	Not disclosed
Baylor College of Medicine	03690011 (2018, U.S.)	T-cell acute lymphoblastic leukemia	*CD7* (Cas9 and gRNA)	Ex vivo (CD7 CAR T cell)	Not disclosed
Peking University	02863913 02867345 02867332 (2016, China)	Bladder cancer Prostate cancer Renal-cell cancer	*PD-1* (Cas9 and gRNA)	Ex vivo (T cells)	Not disclosed

* A search of ClinicalTrials.gov revealed 14 concluded or ongoing trials (NCTs) involving zinc-finger nucleases (ZFNs), 8 past or ongoing NCTs involving transcription activator–like effector nucleases, and 12 past or ongoing NCTs involving clustered regularly interspaced short palindromic repeats associated with Cas9 endonuclease (CRISPR–Cas9). Most of the preclinical work involving ZFNs has been published and peer reviewed, whereas close to none of the preclinical work involving CRISPR–Cas9 has been published or made available for peer review. *ALB* denotes the gene encoding albumin, CAR chimeric antigen receptor, gRNA guide RNA, HIV human immunodeficiency virus, HPV human papillomavirus, HSPC hematopoietic stem and progenitor cell, NY-ESO New York esophageal squamous-cell carcinoma, and TALEN transcription activator–like effector nuclease.

† *BCL11A* is edited at an erythroid enhancer rather than the coding region of the gene.

‡ This trial was a preclinical trial.

§ This trial was a clinical trial.

quence of any small potential change made by a nuclease.

The use of animal models to predict the safety of genetic engineering has not been an effective means of predicting safety in clinical trials. Although genetically engineered cells have been transplanted into immunodeficient mice to ascertain safety,[41] this method cannot be relied on to identify safe or toxic genetic-engineering strategies. Developing animal models is useful, but only if they are time- and cost-efficient and can be shown to reliably predict the results in human clinical trials. Currently, the best approach to evaluating safety is in carefully controlled phase 1 human clinical trials, which not only incorporate standard measures for adjudicating adverse events but also build in analytic studies for the purpose of assessing specific toxic effects associated with genome editing, including clonality and the development of toxic immune responses.

APPLICATIONS TO THE TREATMENT OF HUMANS

MONOGENIC DISEASES

For organ systems such as the hematopoietic and immune systems, the high frequency of gene correction achieved across a range of gene targets for diseases such as sickle cell disease, X-linked severe combined immunodeficiency, and X-linked chronic granulomatous disease is above the therapeutic threshold that is predicted to be curative. These systems will become the subject of clinical trials in the next several years. Hundreds of genetic diseases of the hematopoietic and immune system could, in principle, be cured with the use of this platform. Although monogenic diseases of other organ systems can also be genetically "fixed" through genome editing, challenges remain, including the isolation, expansion, and transplantation of tissue-specific stem cells (for ex vivo therapy) and the delivery of the genome-editing machinery to affected tissues (for in vivo therapy[65,66]).

IMMUNO-ONCOLOGY

Genome editing is being used in clinical trials for the purpose of improving CAR T-cell therapy (ClinicalTrials.gov numbers, NCT02808442 and NCT02746952; see Table 1)[30] and can be used in a number of other ways. NHEJ-mediated editing, which is used to remove the alloreactivity of T cells by knocking out *TCRA* (which encodes TCRα), could also be used to remove immunogenicity by knocking out *B2M* (which encodes β2-microglobulin) and perhaps to increase the potency of cells by removing molecules that inhibit their function or accelerate their exhaustion. HDR-mediated editing can be used to ensure that genes are inserted into a specific locus.[39]

REGENERATIVE MEDICINE

One generally unrealized promise of cell-based therapies is the use of cells or stem cells to replace or restore diseased, damaged, or aging tissue. Genome editing provides a method of engineering cells to increase their potency and safety. Examples of combining regenerative medicine with genome editing include engineering cells to secrete protective factors that prevent neurodegeneration and providing safety switches that readily eliminate cells if they start to cause harm.

SYNTHETIC BIOLOGY

Synthetic biology involves engineering a cell to perform a function it does not normally have. It is now possible to genetically edit cells to secrete therapeutic proteins and to use those cells to influence the physiology of an animal. Examples of combining genome editing and synthetic biology include engineering cells to secrete erythropoietin[73] or wound-healing growth factors.[74] It may become possible to engineer cells to divide, migrate, respond, signal, and secrete in ways that are therapeutically useful to the environment of diseased tissues.

HUMAN GENOME EDITING — HOW TO APPLY?

In 2017, the international study committee convened by the National Academies of Sciences, Engineering, and Medicine concluded that with appropriate regulatory oversight (which does not currently exist in all countries), the use of human genome editing in the study of very early human development would be likely to yield important and unexpected knowledge and should proceed but that its use for enhancement (the creation of traits in healthy humans unrelated to the treatment or prevention of serious disease) should not be pursued.[75] In contrast with previ-

ous assessments, the committee concluded that genome editing that resulted in the transmission of the edit to future generations (heritable or germline editing) might be viewed as acceptable under certain very specific circumstances.[75] The criteria for the possible use of heritable genome editing are strict, have not yet been met, will be challenging to satisfy, and should be interpreted as a functional moratorium. In the United Kingdom, the Nuffield Council on Bioethics released a report with conclusions similar, though not identical, to those of the international study committee.[76] There is a broad consensus that ongoing inclusive and transparent discussion of the applications of genome editing to humans is critical.

The need for transparency and many of the other criteria proposed by the international study committee and the Nuffield council were violated by the unethical application of genome editing to embryos subsequently born as twins, as reported at the Second International Summit on Human Genome Editing in November 2018 in Hong Kong. No report on the case of the twins has been published, and the outcome has not been verified. In any case, even if the twins develop with no adverse events, the work was irresponsible and reckless and violated broad international norms regarding the application of genome editing to human embryos. It highlights the urgency of developing international standards that can be referred to and used to deter such unethical and irresponsible applications from occurring in the future.

CONCLUSIONS

Genome editing represents a transformative means of generating medicines and gives the engineering of the genome a precision that has not previously been possible. Nonetheless, it is a nascent technology, and it is prudent to first apply it in patients with serious conditions. In early phase 1 and phase 2 clinical trials in humans, it will be important to pay attention to details that cannot be explored in preclinical studies and that may facilitate an iterative approach to improving the molecular process involved in genome editing. Once this process has been established as safe and efficacious, its application to less serious diseases could be considered.

A critical issue associated with the development of gene-editing therapies is the goal of making them broadly accessible. The cost of these therapies is likely to be extremely high initially, but cost-benefit analyses, including the cost of care over the lifetime of a patient, may provide justification for their use. Nonetheless, it will be important to control costs to improve equality of access, and continued attention to strategies such as prevention through genetic counseling will remain important.

An audio interview with Dr. Porteus is available at NEJM.org

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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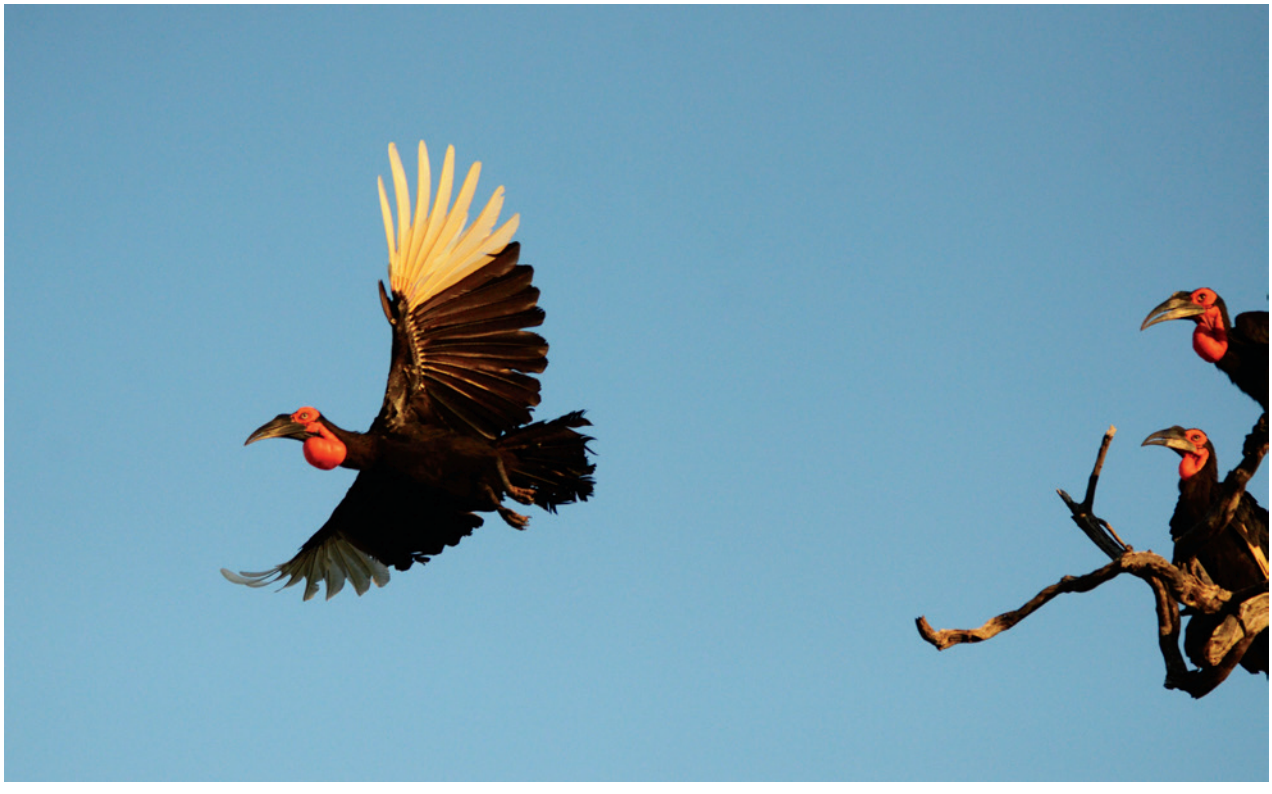

Chobe National Park, Botswana

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