

Gene editing and its impact on the future of clinical medicine

Edycja genów i jej wpływ na przyszłość medycyny klinicznej

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Abstract:

Genetic mutations lead to multiple diseases that affect millions of people worldwide. Since the discovery of the double-helix DNA structure in 1953 by Franklin, Crick and Watson, a lot of research has been conducted to study the technologies that could edit the genetic code. Molecular tools such as ZFNs, TALENs and CRISPR-Cas9 have been developed to modify DNA. ZFNs are programmable and synthetic nucleases made of zinc finger DNA-binding domains fused to a FokI nuclease, and they are capable of making double-stranded breaks in targeted genes. TALENs are proteins that were engineered. They combine TALE DNA-binding domains with a FokI nuclease. They can recognise a specific DNA sequence and then cleave through the double helix. CRISPR-Cas9 technology is based on a bacterial defence mechanism. It utilises a guide RNA to direct the Cas9 nuclease to a specific DNA sequence, where it cleaves the sequence, which is later repaired via cellular mechanisms. All methods have pros and cons, and all of them also need further research to establish how safe they are to eliminate their limitations and eventually use them for personalised medicine. Gene editing tools can be used in various medical fields, such as infectious diseases, hereditary diseases or oncology. However, the application of molecular tools in clinical medicine raises a lot of ethical concerns, such as safety, privacy, consent and the impact of gene editing on society, and potentially risks increased

social inequality. There is also a lack of legal guidelines that would specify how gene editing can be used in a clinical setting.

Keywords: gene editing, CRISPR-Cas9, ZFNs, personalised medicine, TALENs

Streszczenie:

Mutacje genetyczne prowadzą do wielu chorób, które dotyczą miliony ludzi na całym świecie. Od odkrycia struktury podwójnej helisy DNA w 1953 roku przez Franklina, Cricka i Watsona przeprowadzono wiele badań w celu stworzenia technologii, które mogłyby edytować kod genetyczny. Narzędzia molekularne, takie jak ZFNs, TALENs i CRISPR-Cas9, zostały opracowane w celu edycji DNA. ZFNs to programowalne i syntetyczne nukleazy zbudowane z domen wiążących DNA typu „zinc finger” połączonych z nukleazą FokI, które są zdolne do tworzenia dwuniciowych pęknięć w docelowych genach. TALENs to białka, które zostały zaprojektowane inżynieriynie, łączące domeny wiążące DNA TALE z nukleazą FokI. TALENs potrafią rozpoznać określoną sekwencję DNA, a następnie przeciąć podwójną helisę. Technologia CRISPR-Cas9 opiera się na mechanizmie obronnym bakterii. Wykorzystuje guide RNA, aby skierować nukleazę Cas9 do określonej sekwencji DNA, gdzie przecina sekwencję, która jest później naprawiana za pomocą mechanizmów komórkowych. Wszystkie metody mają zalety i wady, oraz wszystkie z nich wymagają dalszych badań, aby ustalić, jak są bezpieczne, wyeliminować ich ograniczenia i ostatecznie wykorzystać je w medycynie spersonalizowanej. Narzędzia do edycji genów mogą być stosowane w różnych dziedzinach medycyny, takich jak choroby zakaźne, choroby dziedziczne lub onkologia. Stosowanie narzędzi molekularnych w medycynie klinicznej jednak budzi wiele obaw etycznych, takich jak bezpieczeństwo, prywatność, świadoma zgoda i wpływ edycji genów na społeczeństwo, a także potencjalnie zwiększa ryzyko nierówności społecznych. Brakuje również wytycznych prawnych, które określałyby, w jaki sposób edycja genów może być stosowana w warunkach klinicznych.

Słowa kluczowe: edycja genów, CRISPR-Cas9, ZFNs, medycyna personalizowana, TALENs

Introduction

Since *Photo 51* – an X-ray image of the deoxyribonucleic acid (DNA) fibres taken by Rosalind Franklin – and the discovery of the double-helix DNA structure in 1953 by Crick and Watson, a lot of research has been conducted to develop gene editing technologies. To date, approximately 6000 to 8000 hereditary diseases have been defined (1). However, the development of molecular research and the continuous improvement of molecu-

lar tools have expanded the application of gene-editing technologies beyond hereditary disorders. More than 250 new and rare hereditary disorders are described every year, and gene editing technologies are progressively being explored not only for the treatment of genetic diseases but also for the treatment of, inter alia, infectious diseases and cancer (1). Thus, the role of gene editing in clinical medicine has received increased attention across several medical specialities in recent years. Methods such as TAL effector nucleases (TALENs) (2), zinc finger nucleases (ZFNs) (3), and CRISPR-Cas9 (4) have dramatically changed and modernized genetic engineering. The introduction of new genes or repairing faulty genes facilitates the modification of the genome by targeted genome editing (5). Precise medicine approach to patients heavily relies on genetic engineering that is used to create animal and cell models of human diseases that are infectious or hereditary as well as cancers. Disease models allow an analysis of molecular and cellular bases of pathogenesis that contribute to the development of potential drugs and/or treatment targets (6). With the development of molecular tools DNA editing progressed from the use of mutagens such as chemical or physical agents to modify DNA to the use of engineered tools that are capable of creating gene knock-in and knock-out (5). The rapid development of medicine requires modern therapeutic tools, and genome editing technologies offer a unique opportunity to treat diseases at their genetic root, thereby justifying the use of genetic editing tools in clinical practice. However, gene editing and molecular research raise a lot of ethical questions. In the literature on gene editing, the relative importance of ethical considerations has been subject to considerable discussion. Issues such as genetic privacy, informed consent and the implications of using molecular tools are the main concerns (7). This paper analyses the three types of molecular tools mentioned above and how the use of the older methods, such as TALENs, ZFNs and the newer method – CRIPR-Cas9 can potentially affect the future of clinical medicine. Moreover, the ethical concerns of gene editing are going to be discussed too.

Methodology

A comprehensive search was conducted using databases with academic publications, such as PubMed and Google Scholar. Keywords used for the search of relevant literature include “gene editing”, “CRISPR-Cas9”, “ZFNs”, “personalised medicine”, and “TALENs”. Boolean operators such as AND and OR were applied to improve search results. Articles were included if they were published between 2012 and 2024 with the exception of two articles – one published in 2005 and the other one in 2009. The articles published outside of the selected time frame were included because of the important information they

provide. The inclusion criteria for publications were reviews, meta-analysis and original research papers, all written in English, focusing on gene editing tools and their mechanism as well as their applications in disease research and therapy. The exclusion criteria were publication without full-text availability, non-peer-reviewed publications, and materials published outside of the selected time frame. Findings, methodology and key themes were extracted from all included sources.

Molecular tools

Zinc finger nucleases

ZFNs are programmable nucleases that were commonly used in past genome-editing research. Nowadays, other methods are used more often that require less labour to achieve similar results. ZFNs are made of synthetic enzymes conceding at least three zinc finger domains. Each domain binds 3 bp of DNA, and when connected, they generate an artificial DNA binding protein which binds ≥ 9 bp of DNA (8). One-half of the FokI nuclease bonds to the zinc finger domains in a matter that when two ZFNs bind the two distinctive 9 bp sites that are divided by a spacer are capable of cleaving within the spacer to create double-strand breaks (DSB) (8). Originally, when this method was developed, naturally occurring zinc finger domains were used to detect the DNA sequence in the genome of the *Escherichia coli* bacteriophage lambda (8). Results from further studies demonstrate that ZFNs can be programmed to target fundamentally any target site in the human genome. Moreover, it results in a precise repair of the mutations in the interleukin-2 receptor [gamma] chain gene by homology-directed repair (HDR) in $\sim 20\%$ of transfected cells (9). Figure 1 provides a visual representation of this mechanism. Various proof-of-concept studies have been conducted using this method for gene editing. According to Geurts, gene-targeted animals can be created with ZFNs by editing embryos that were fertilized in vitro 10. This approach of DNA editing was later used to model human diseases by various researchers in, inter alia, rodents (11) and zebrafish (12), to understand the pathology of the diseases. According to a study from 2017, gene editing displayed a real therapeutic potential in vivo, as haemostasis was restored following the repair of a mutant F.IX gene in a mouse model of haemophilia B. Furthermore, this method can possibly correct various disease-causing mutations that occur within the same gene if ‘superexon’ is used (8). However, ZFNs have a couple of limitations. The ratio of off-target mutations is relatively high. Because each ZFN can recognise only 3 bp, unintentional mutations can occur at sites that are relatively similar to the target site because of an

exhibition of similar sequences. Moreover, immunogenicity can occur in the host body during the use of ZFNs, which are considered a foreign body. ZFNs can cause a cell-mediated or humoral immune response in the host organism (13).

TAL effector nucleases

Developed in 2010, TAL effector nucleases also referred to as TALENs is another method of gene editing that followed in the footsteps of ZFNs but as a method, TALENs are easier to execute. They can be used for modification in crops, livestock, non-model and model organisms (2). In 2015 it was used to cure cancer in humans (14). TALENs are a combination of activator-like effectors (TALE) and the catalytic domain of the restriction endonuclease FokI. TALEs are plant proteins that come from pathogenic *Xanthomonas* bacteria. When TALEs are injected into cells from plants they can locate the nucleus and bind to target promoters and as a result, induce gene expression. TALE proteins have three functional domains. The C-terminal (the end of the protein) domain contains an interaction interface for the plant transcription factor IIA, two functional nuclear localization signals, and an acidic activation domain (15). The N-terminal (the beginning of the protein) contains the bacterial secretion signal and a non-specific DNA-binding activity (16). Those are necessary to create the affinity of the protein to DNA (16). The central repeat section of TALE is key to the programmable DNA binding. The central repeat region is usually made of 33-35 amino acid-long tandem repeats and the acids that are located at positions 12 and 13 of each repeat are the ones that define the DNA specificity of the TALE, the repeat-variable di-residues (RVD) (17). All possibilities of RVD combinations have been decoded (18). The evidence shows that unique/specific combinations detect only a single nucleotide. Furthermore, more flexible combinations can recognize two, three or all four nucleotides (19). Because of that DNA binding attributes of TALENs can be adjusted as desired via re-arrangement of the repeats (14). According to further single-molecule research, it was discovered that TALEs search for DNA target sites by wrapping around DNA and performing a quick, one-dimensional, non-rotational and unbiased search (19, 20). Figure 1 provides a visual representation of this mechanism. TALENs have been successfully used to cure a human patient from cancer via genome editing in 2015 (21). Cellectis, a French biopharmaceutical company, used TALENs to inactivate genes that trigger non-self-immune reactions from immune cells. A foreign person donated their cells as a source for the gene transfer to create chimeric antigen receptor T lymphocyte cells (CAR-T immune cells) (21). Those cells edited via TALENs were used to eliminate tumour cells and treat two individuals from leukaemia who were

11 and 16 months old (21). This therapy has proven real-life implications in medicine and the effects of the use of gene editing tools in patients. However, one of the limitations of TALENs is that it is significantly bigger than for example, ZFNs. This means the delivery of TALENs to targets is not as express and it could be especially when using vectors such as viral one with size limitation. Moreover, since TALENs need a specific sequence at the target it can limit the number of potential target sites. For example, a thymine nucleotide must come before the target region's sequence (22).

CRISPR-Cas9

Clustered regularly interspaced short palindromic repeats (CRISPR) – CRISPR-associated (Cas) system is a natural process. It is a bacterial protection mechanism against phage infection and plasmid transfer. However, it has been converted to a ribonucleic acid-guided (RNA-guided) DNA targeting tool for gene editing, epigenetic mutation, transcriptional perturbation, and genome imaging (23). CRISPR-Cas9 has revolutionized the biotechnology field due to its precise gene editing capabilities. The two main components are: clustered regularly interspaced short palindromic repeats and Cas proteins responsible for cleaving the DNA (23, 24). In a bacterium, when it is invaded by a virus, the Cas protein will cut out a segment of the virus's DNA and attach it to the bacteria's CRISPR region seizing a molecular outline of the infection. Next, the virus's DNA is copied into short pieces of RNA. In CRISPR, the role of RNA is to bind to Cas9. It results in the creation of a complex that latches onto free-floating genetic material and scouts for a match to the virus (25). When the virus infects the bacteria again, it is recognized instantly by the complex of RNA and Cas9. As a result, Cas9 exterminates the viral DNA (23). In 2012, it was established how to use CRISPR to target any DNA in any organism and, it became a gene editing tool. The mechanism of CRISPR-Cas-9 genome editing: recognition, cleavage, and repair (25). To edit a chosen gene, a guide RNA is designed to match the target gene. Then, the RNA is attached to Cas9. The guide RNA leads Cas9 to the target gene and, Cas9 cleaves the DNA. In other words, via an injection of Cas9 that is bound to a custom guide RNA essentially any gene can be edited in the genome (24). After the DNA is cleaved the cell will try to repair it. Proteins called nucleases are responsible for trimming the broken ends and joining them together. This process is called nonhomologous end joining (NHEJ). However, if a mistake occurs during that process there can be an extra or missing base making the target gene unusable or turned off (23). Thus, a distinct sequence of DNA templates is added to CRISPR to trigger the proteins to perform homology-directed repair and use the DNA template as an instruction to the repair process of the target gene (24). Figure 1 provides

a visual representation of this mechanism. This method can be used to create new treatment methods for diseases strictly caused by genetic mutations such as blood disorders like sickle or genetic disorders such as sickle cell anaemia (26), cystic fibrosis (27), and Duchene Muscular Dystrophy (28) and potentially neurological disorders such as Huntington's Disease (29). Exagamglogene autotemcel therapy which is based on CRISPR-Cas9 has been approved in Great Britain and the United States of America in 2023 (30). In Great Britain, exagamglogene autotemcel therapy can be used in patients with transfusion-dependent β Thalassemia and in patients with sickle cell anaemia aged 12 years or older who need hematopoietic stem cell transplantation but lack a suitable donor (30). In the United States, exagamglogene autotemcel can be used in sickle cell anaemia patients aged ≥ 12 years with recurrent vaso-occlusive crises and in patients with transfusion-dependent β Thalassemia (30). Moreover, it is a tool to create genetic models to study the human genome and observe what happens when certain genes are, for example, silenced or modified. However, there are some concerns regarding the routine use of CRISPR-Cas9 such as the toxic and immune response of treated cells to CRISPR-Cas9 components. Furthermore, further research is necessary to establish high-specific guide RNAs and use high-specificity Cas enzymes to limit the off-target impact (31).

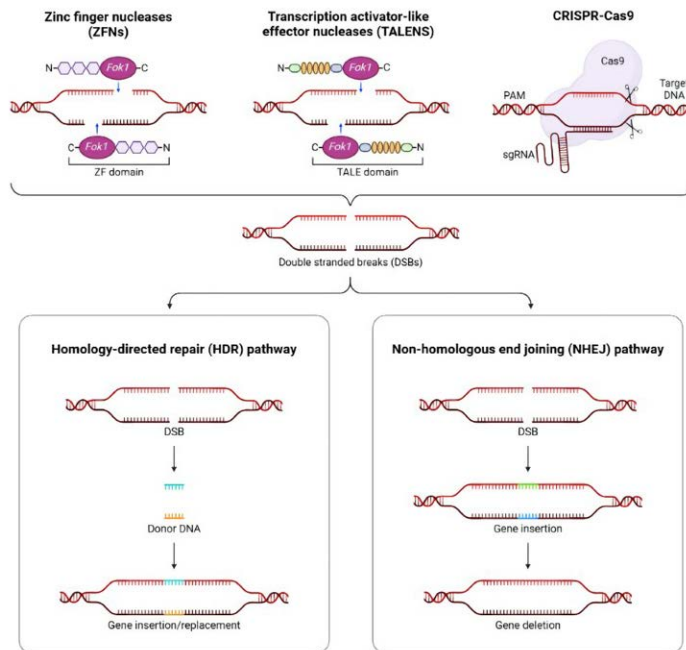


Figure 1. Comparison of mechanisms of action of genome editing tools: ZFNs, TALENs and CRISPR-Cas9 and repair pathways. Obtained from BioRender.com

The future of clinical medicine and gene editing

According to CRISPR Medicine News from January 2025, in 2024 239 gene-editing or gene-editing therapeutic studies were identified and 152 of them at that time were active including recruiting, not yet recruiting and enrolling by invitation. Those studies were dominated by therapeutic candidates for blood and solid cancers making up almost half of all trials (32).

Various fields in medicine can benefit from gene editing tools and create new treatments and therapies for affected patients. One of the fields in infectious diseases. Molecular tools could be used for the treatment by using them to remove viruses' genetic material that is integrated into the host's cell's genome (6). The second approach to infectious diseases can be a modification of the host's cellular receptors that viruses use to enter the host's cells (6). This could be applied specifically to human immunodeficiency virus (HIV). Encoding chemokine receptor 5 (*CCR5*) is a protein located in T cells. HIV binds to *CCR5* and it is a critical co-receptor for HIV entry into host cells alongside CD4 (33). An experiment has been carried out on two embryos using CRISPR-Cas9 to remove the gene from their genome and prevent HIV infection. The experiment was conducted unethically and illegally in China (34). However, there is a potential for the knock-out approach to prevent viral infections.

The hereditary diseases field is another category of medicine that can benefit from the use of gene editing tools (6). Genetic mutations can lead to the acquisition or loss of function of the mutated genes. In the case of gain of harmful function in autosomal dominant disorder such as achondroplasia (a bone growth disorder), creating a double-stranded break in the affected gene allele would lead to a deletion or insertion post nonhomologous end joining process (NHEJ) resulting in a frameshift and shortened protein that would not modify the patient's phenotype (35). Another solution for correcting the kind of pathogenic variant is restoring the wild-type phenotype via the induction of homologous recombination (35). Hereditary diseases that are characterised by prolonged short tandem repeats (STR) could be potentially corrected by a two-site cleave on both sides of the sequence. This approach could be used to delete it from the gene allele. Moreover, in the case of STR producing a deleterious protein that disturbs normal cellular functions, induction of NHEJ could be used to remove the harmful protein. So far, this approach has been investigated for Huntington's disease (36).

Another branch of hereditary diseases is autosomal and X-linked recessive disorders. Those diseases are more complex because both alleles have pathogenic mutations (37).

In that case, using the non-homologous end-joining method will not be effective because it would cause a loss of protein function. Thus, a programmable endonuclease system a donor DNA portion (crucial for homologous recombination) and an unmodified gene sequence are incorporated into the cell. Then, they are used by proteins responsible for the HR process to reconstruct the pathogenic variants (38). Furthermore, certain recessive disorders may benefit from the eradication of exon/exons that have premature stop codons and restoring most of the protein sequence and partially restoring the function of the protein according to research performed on cells obtained from patients with Duchenne muscular dystrophy (39).

Another field of medicine that can benefit from gene editing is oncology, specifically the treatment of malignant tumours. During the last decade, the link between T cells and their use for cancer treatment has been at the centre of much attention. Results from a previous study demonstrate that chimeric antigen receptor T lymphocyte cells (CAR-T cells) therapy could be used for cancer treatment (40). The principle of this therapy is to create T lymphocytes that are capable of recognising with precision and then fighting cancer cells. To develop those cells, T lymphocytes of cancer patients are adoptively transferred with chimeric protein receptor genes that are expressed by malignant cells; therefore, they are recognised and destroyed by immune cells. Genetically engineered T cells are called ‘living drugs’ (40, 41). However, T cells could undergo a few changes to make them more viable. To prevent graft-versus-host reaction, the T cell genome can be edited via the inactivation of the genes coding the T cell receptor. Moreover, to reduce immunogenicity, T cells can be edited to eliminate HLA-I antigens. Changes in the T cell receptor and HLA-I elimination give scientists the opportunity to create all-encompassing CAR-T cells that could be used for the treatment and management of multiple types of tumours (42, 43).

Ethical consideration

Gene editing and the use of molecular tools in medicine raise a lot of questions. One of the main concerns is editing the human germline, who would have access to that, and how it would affect future generations and the lives of those who underwent this procedure. Since gene editing is a relatively new science, there are also not many legal definitions and policies of those treatments and how they can be used (6). As mentioned in the section above, a germline editing experiment has already been carried out in China by Jiankui He in 2018, and two twin sisters were born. He was immediately criticised

for his unprofessional approach and illegal practice after presenting the details of his investigation during the Second International Summit of Human Genome Editing (6). The long-term effects of his actions are currently unknown. Moreover, since future generations cannot consent to heritable genetic modifications, we cannot predict at his moment how this modification will affect the offspring of the twin girls (44). According to the statement of The American Society for Human Genetics from 2017, at this time, considering the number of unanswered scientific, ethical and policy questions, germline editing should not be performed (45). Furthermore, it is also discussed who and how will be able to access those tools. Although sequencing and gene editing are getting cheaper, people from low-income countries may be excluded from the opportunity to access those tools; therefore, one of the ethical questions is whether gene editing will cause further social inequalities (45). Also, as of yet, we are aware that some of the molecular tools can still lead to off-target effects that could cause unpredictable and harmful effects that could potentially be heritable for the next generations (45). Further research is required to understand in depth the complex side effects of gene editing and with the progressive development of gene editing technologies, it is crucial to balance academic and scientific pursuits with the possible impact on the human race.

Conclusions

The number of diseases caused by genetic mutations highlights the need for molecular tools that would enable more effective and efficient modelling and treatment of those diseases. Genetic engineering tools such as zinc finger nucleases, TAL effector nucleases and CRISPR-Cas9 have changed molecular engineering, allowing us to model diseases to understand the pathological mechanism as well as allow us to use a personalised medicine approach to treat patients with genetic disorders. Although those technologies have much potential, each method has potential side effects, and, in general, modifying human DNA raises a lot of ethical and legal questions. ZFNs have therapeutic potential and can correct mutations, such as, in haemophilia B, and they are also used to model diseases in animals. However, the drawbacks are that there is a relatively high chance of off-target mutation and the risk of an immune response against the proteins used in this method since ZFNs are recognised as outsider proteins. TALENs have been successfully used before in clinical medicine for the treatment of cancer patients via engineered immune cells. However, the limitation is that TALENs are large, which makes their delivery challenging. Moreover, they additionally need a specific DNA sequence to narrow the target sites. CRISPR-Cas9 is the latest molecular tool that uses a bacterial defence mechanism

for cleaving and editing DNA. This technology uses the Cas9 protein and programs it to target a specific sequence. It is a groundbreaking method, but the drawback is the risk of unintended off-target mutations and the possibility of an immune response to the Cas9 protein. In clinical medicine, gene editing can be used in various fields such as infectious diseases, hereditary diseases or oncology. However, the main ethical questions cover concerns such as privacy, consent of current and future generations, availability and accessibility of those methods and social impact as well as safety concerns. Currently, there are not enough legal frameworks and consensus on who should access and how gene editing could be used daily in clinical medicine. Further research is necessary to improve the tools before applying them in medical practice.

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Wiera Magdalena Tolczyk: conceptualization, study design, data collection, data analysis, writing – original draft; Gabriela Maria Piech: data collection, writing – original draft; Igor Lis: data collection, writing – original draft.

Abbreviations:

DNA – deoxyribonucleic acid

TALENs – TAL effector nucleases

ZFNs – zinc finger nucleases

DSB – double strands breaks

HDR – homology-directed repair

TALE – activator-like effector

RVD – repeat-variable di-residues

CRISPR – clustered regularly interspaced short palindromic repeats

RNA – *ribonucleic acid*

STR – short tandem repeats

NHEJ – nonhomologous end joining

CAR-T – chimeric antigen receptor T lymphocyte cell

CCR5 – encoding chemokine receptor 5

HIV – human immunodeficiency virus

Bibliografia:

1. Dawkins, H.J.S. *et al.* (2017) ‘Progress in rare diseases research 2010–2016: An IRDIRC perspective’, *Clinical and Translational Science*, 11(1), pp. 11–20. doi:10.1111/cts.12501.
2. Becker, S. and Boch, J. (2021) ‘Tale and TALENs genome editing technologies’, *Gene and Genome Editing*, 2, p. 100007. doi:10.1016/j.ggedit.2021.100007.
3. Ochiai, H. and Yamamoto, T. (2023) ‘Construction and evaluation of zinc finger nucleases’, *Methods in Molecular Biology*, pp. 1–25. doi:10.1007/978-1-0716-30167_1.
4. Hendriks, D., Clevers, H. and Artegiani, B. (2020) ‘CRISPR-cas tools and their application in genetic engineering of human stem cells and organoids’, *Cell Stem Cell*, 27(5), pp. 705–731. doi:10.1016/j.stem.2020.10.014.
5. Pandita, D. and Pandita, A. (2023) *CRISPR*. Palm Bay, FL, Boca Raton, FL: AppleAcademic Press Inc. ; CRC Press.
6. Petraitytė, G., Preikšaitienė, E. and Mikštienė, V. (2021) ‘Genome editing in medicine: Tools and challenges’, *Acta medica Lituanica*, 28(2), p. 8. doi:10.15388/amed.2021.28.2.8.
7. Pandey, P. (2024) ‘Balancing Innovation and Responsibility: The ethical and societal impacts of ai in the internet age’, *International Journal of Science and Research(IJSR)*, 13(12), pp. 972–973. doi:10.21275/sr241121232016.
8. Harrison, P.T. and Hart, S. (2018) ‘A beginner’s Guide to gene editing’, *Experimental Physiology*, 103(4), pp. 439–448. doi:10.1113/ep086047.
9. Komiyama, M. (2005) ‘Faculty opinions recommendation of highly efficient endogenous human gene correction using designed zinc-finger nucleases.’, *Faculty Opinions – Post-Publication Peer Review of the Biomedical Literature*[Preprint]. doi:10.3410/f.1025053.324988.
10. Geurts, A.M. *et al.* (2009) ‘Knockout rats via embryo microinjection of zinc-fingernucleases’, *Science*, 325(5939), pp. 433–433. doi:10.1126/science.1172447.
11. Birling, M.-C., Herault, Y. and Pavlovic, G. (2017) ‘Modeling human disease in rodents by CRISPR/ Cas9 genome editing’, *Mammalian Genome*, 28(7–8), pp. 291301. doi:10.1007/s00335-017-9703-x.
12. Morales, E.E. and Wingert, R.A. (2017) ‘Zebrafish as a model of kidney disease’, *Results and Problems in Cell Differentiation*, pp. 55–75. doi:10.1007/978-3319-51436-9_3.
13. Benhabiles, H., Jia, J. and Lejeune, F. (2016) ‘Strategies to correct nonsense mutations’, *Nonsense Mutation Correction in Human Diseases*, pp. 107 165. doi:10.1016/b978-0-12-804468-1.00003-8.
14. Qasim, W. *et al.* (2017) ‘Molecular remission of infant B-all after infusion of Universal TALENs gene-edited car T cells’, *Science Translational Medicine*, 9(374).doi:10.1126/scitranslmed.aaj2013.

15. (2016) *Decision letter: A host basal transcription factor is a key component for infection of rice by tale-carrying bacteria* [Preprint]. doi:10.7554/elife.19605.031.
16. Chai, J., Han, Z. and Gao, H. (2012) *Crystal structure of a tale protein reveals an extended N-terminal DNA binding region* [Preprint]. doi:10.2210/pdb4hpz/pdb.
17. Boch, J. *et al.* (2009) 'Breaking the code of DNA binding specificity of tal-type III effectors', *Science*, 326(5959), pp. 1509–1512. doi:10.1126/science.1178811.
18. Yang, J. *et al.* (2014) 'Complete decoding of tal effectors for DNA recognition', *Cell Research*, 24(5), pp. 628–631. doi:10.1038/cr.2014.19.
19. Miller, J.C. *et al.* (2015) 'Improved specificity of tale-based genome editing using an expanded RVD repertoire', *Nature Methods*, 12(5), pp. 465–471. doi:10.1038/nmeth.3330.
20. Cuculis, L. *et al.* (2016) 'Tale proteins search DNA using a rotationally decoupled mechanism', *Nature Chemical Biology*, 12(10), pp. 831–837. doi:10.1038/nchembio.2152.
21. Qasim, W. *et al.* (2017a) 'Molecular remission of infant B-all after infusion of Universal TALENs gene-edited car T cells', *Science Translational Medicine*, 9(374). doi:10.1126/scitranslmed.aaj2013.
22. Nemudryi, A.A. *et al.* (2014) 'TALENs and CRISPR/Cas genome editing systems: Tools of discovery', *Acta Naturae*, 6(3), pp. 19–40. doi:10.32607/20758251-2014-6-3-19-40.
23. Jiang, F. and Doudna, J.A. (2017) 'CRISPR–cas9 structures and mechanisms', *Annual Review of Biophysics*, 46(1), pp. 505–529. doi:10.1146/annurev-biophys-062215010822.
24. Mengstie, M.A. and Wondimu, B.Z. (2021) 'Mechanism and applications of CRISPR/cas-9-mediated genome editing', *Biologics: Targets and Therapy*, Volume 15, pp. 353–361. doi:10.2147/btt.s326422.
25. Jinek, M. *et al.* (2012) 'A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity', *Science*, 337(6096), pp. 816–821. doi:10.1126/science.1225829.
26. CRISPR-Cas9 gene editing for sickle cell disease and β -thalassemia' (2021) *New England Journal of Medicine*, 384(23). doi:10.1056/nejmc2103481.
27. Da Silva Sanchez, A. *et al.* (2020) 'Treating cystic fibrosis with mrna and CRISPR', *Human Gene Therapy*, 31(17–18), pp. 940–955. doi:10.1089/hum.2020.137.
28. Happi Mbakam, C. *et al.* (2022) 'CRISPR-Cas9 gene therapy for Duchenne muscular dystrophy', *Neurotherapeutics*, 19(3), pp. 931–941. doi:10.1007/s13311-022-01197-9.
29. Alkanli, S.S. *et al.* (2022) 'CRISPR/Cas9 mediated therapeutic approach in Huntington's disease', *Molecular Neurobiology*, 60(3), pp. 1486–1498. doi:10.1007/s12035-022-03150-5.
30. Hoy, S.M. (2024) 'Exagamglogene Autotemcel: First approval', *Molecular Diagnosis & Therapy*, 28(2), pp. 133–139. doi:10.1007/s40291-024-00696-z.
31. Zhang, B. (2020) 'CRISPR/Cas Gene therapy', *Journal of Cellular Physiology*, 236(4), pp. 2459–2481. doi:10.1002/jcp.30064.
32. News: CRISPR medicine in 2024 – A recap (no date) CRISPR Medicine. Available at: https://crisprmedicine.com/news/crispr-medicine-in-2024-a-recap/?utm_source=chatgpt.com (Accessed: 29 May 2025).
33. BreLOT, A. and Chakrabarti, L.A. (2018) 'CCR5 revisited: How mechanisms of HIV entry govern AIDS pathogenesis', *Journal of Molecular Biology*, 430(17), pp. 25572589. doi:10.1016/j.jmb.2018.06.027.

34. Mills, P. (2019) 'Preimplantation genome editing: *ccr5* in China', *Emerging Topics in Life Sciences*, 3(6), pp. 695–700. doi:10.1042/etls20190114.
35. Miao, K. *et al.* (2019) 'Optimizing CRISPR/cas9 technology for precise correction of the FGFR3-G374R mutation in achondroplasia in mice', *Journal of Biological Chemistry*, 294(4), pp. 1142–1151. doi:10.1074/jbc.ra118.006496.
36. Ekman, F.K. *et al.* (2019) 'CRISPR-Cas9-mediated genome editing increases lifespan and improves motor deficits in a huntington's disease mouse model', *Molecular Therapy – Nucleic Acids*, 17, pp. 829–839. doi:10.1016/j.omtn.2019.07.009.
37. Lukin, J., Smith, C.M. and De Rubeis, S. (2024) 'Emerging X-linked genes associated with neurodevelopmental disorders in females', *Current Opinion in Neurobiology*, 88, p. 102902. doi:10.1016/j.conb.2024.102902.
38. Tang, X.-D. *et al.* (2019) 'Methods for enhancing clustered regularly interspaced short palindromic repeats/cas9-mediated homology-directed repair efficiency', *Frontiers in Genetics*, 10. doi:10.3389/fgene.2019.00551.
39. Li, H.L. *et al.* (2015) 'Precise correction of the dystrophin gene in Duchenne muscular dystrophy patient induced pluripotent stem cells by TALENs and CRISPR Cas9', *Stem Cell Reports*, 4(1), pp. 143–154. doi:10.1016/j.stemcr.2014.10.013.
40. Fesnak, A.D., June, C.H. and Levine, B.L. (2016) 'Engineered T cells: The promise and challenges of cancer immunotherapy', *Nature Reviews Cancer*, 16(9), pp. 566–581. doi:10.1038/nrc.2016.97.
41. Young, R.M. *et al.* (2022) 'Next-generation CAR T-cell therapies', *Cancer Discovery*, 12(7), pp. 1625–1633. doi:10.1158/2159-8290.cd-21-1683.
42. Poirot, L. *et al.* (2015) 'Multiplex genome-edited T-cell manufacturing platform for "off-the-shelf" adoptive T-cell immunotherapies', *Cancer Research*, 75(18), pp. 3853–3864. doi:10.1158/0008-5472.can-14-3321.
43. Liu, Xiaojuan *et al.* (2016) 'CRISPR-Cas9-mediated multiplex gene editing in CAR-T cells', *Cell Research*, 27(1), pp. 154–157. doi:10.1038/cr.2016.142.
44. Rothschild, J. (2020) 'Ethical considerations of gene editing and genetic selection', *Journal of General and Family Medicine*, 21(3), pp. 37–47. doi:10.1002/jgf2.321.
45. Ormond, K.E. *et al.* (2017) 'Human germline genome editing', *The American Journal of Human Genetics*, 101(2), pp. 167–176. doi:10.1016/j.ajhg.2017.06.012.