Narrative Review Article

CRISPR-Cas Technology: Mechanisms, History, and Emerging Clinical Applications

Sansan Lu

Harriton High School, 600 N Ithan Ave, Bryn Mawr, PA19010, United States

ABSTRACT

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated Cas proteins form the CRISPR–Cas system, one of the most prominent genome-editing technologies to date. Originally discovered as an immune system in bacteria against viral infection, the system has since been adapted into a precise genome-editing tool. CRISPR-Cas technology has reshaped modern biology and medicine, with applications spanning basic research, agriculture, and clinical medicine. Promising outcomes include the correction of genetic disorders and the engineering of immune cells for cancer therapy. This review outlines the fundamental mechanisms of CRISPR-Cas technology, traces key milestones in its development, and highlights recent advances in the field. Emerging clinical applications and ongoing trials are also discussed, along with ethical considerations and the importance of appropriate regulatory oversight.

Keywords: Gene editing; CRISPR-Cas Technology; Clinical trials; ethics; breakthroughs

INTRODUCTION

Clustered Regularly Interspaced Short Palindromic Repeats, also known as CRISPR, is one of the most prominent gene editing technologies to date (1, 2). Discovered in microbes as an immune system, CRISPR assists in establishing a memory of a virus in the cell (3). Briefly, when a virus enters the bacterial cell, the cell will integrate a piece of the virus's DNA into its own genome and create a guide RNA using this new sequence. The next time the same virus enters the cell, the guide RNA will recognize the virus's DNA through complementary base pairs, bind to it, and a DNase (DNA-cutting enzyme) will cut the virus DNA (4). Scientists recognized the great potential of this RNA-guided-site-specific-DNA-cut system and engineered it to be used in eukaryotic cells, especially the human cells for disease treatment (5).

But, what can the CRISPR-Cas technology actually do? Most obviously, it is used for treating genetic diseases, many of which, before CRISPR-Cas technology, had no good cure. So far, it is especially good for diseases with a single gene mutation, such as sickle cell anemia. It can also be useful for cell therapy, a practice where genetically engineered cells are imported into a person to help fight a disease (1). For example, CRISPR-Cas technology can offer more control over modified T cells, which, without this technology, may fail to work or start attacking healthy cells along with the infectious ones. Additionally, CRISPR-Cas technology can help with epigenetic therapy (aims to modify the expression of genes without touching the protein-encoding DNA sequences), manufacturing (creating a greater result of

Corresponding author: Sansan Lu, E-mail: sansan.33.lu@gmail.com.

Accepted October 15, 2025
https://doi.org/10.70251/HYJR2348.35893900

products), ecological engineering (bringing back extinct animals or eliminating certain species), diagnostics, sustainability of food and energy, and more (1).

Nevertheless, despite its power, it is important to recognize that the current CRISPR-Cas technology has its limits and drawbacks. It can result in off-target effects, which occur when Cas protein makes cuts on an untargeted segment of the gene. These off-target effects are often connected to mismatch of the previously mentioned gRNA to another DNA region, as Cas9 is known to tolerate up to three mismatches between the gRNA and DNA.

Another limitation of CRISPR-Cas technology is mosaic mutations, meaning that the targeted genomic change is only introduced in some but not all desired cells. This most commonly occurs when CRISPR-Cas technology is used on embryo genetic editing, where cells divide faster than the time that Cas complex takes to complete its task. Other concerns include immune responses triggered by bacterial origins of Cas proteins; or scalability, in which the CRISPR-Cas technology can only make a limited number of edits on the genome for a given round.

Nevertheless, CRISPR-Cas technology is still very promising. This minireview includes the technology's mechanism, brief history, breakthroughs and clinical trials, ethical considerations, and future directions. It aims to provide an accessible overview of the CRISPR-Cas technology for high school students who are interested in genetic editing.

MECHANISMS OF CRISPR-CAS TECHNOLOGY

A huge advantage of the CRISPR-Cas technology is its ability to target the exact part of the DNA that needs to be changed using the guide RNA (gRNA). TracrRNA (trans-activating RNA) and crRNA (CRISPR RNA) together form the gRNA, which help the DNA-cutting enzyme, Cas, to the correct DNA location. The guide RNA recognizes the DNA sequence through the matching nucleotide sequence (6). In addition, a Protospacer Adjacent Motif, or PAM, is required. PAM is a short strand of nucleotide base pairs on the non-target strand of the DNA that tells the Cas protein where to cut the DNA. PAM is different and specific for each DNA cutting enzyme or Cas protein; for Cas9, the PAM sequence is NGG, where 'N' is any nucleotide base followed by two guanines (7). Cas12a—discovered a few years after Cas9 and does not require tracrRNA—has a PAM sequence of TTTV, which are three thymines followed by either adenine, guanine, or cytosine. Once the Cas complex binds to the correct area of the genome, the DNA will be unwound and cut at three-nucleotide upstream of the PAM sequence (8).

After the DNA is cut, there are different outcomes, depending on the design. If the purpose is to use the CRISPR-Cas technology to correct an unwanted or disease-causing genetic mutation, a correct DNA template will be provided at the same time. The process of integrating the correct DNA sequence is called Homology-Directed Repair, or HDR. This is how a lot of gene therapies work: the bad DNA is cut, and a template DNA with the correct sequence will be introduced and replace the bad copy (9). HDR works best in actively dividing cells and will often occur in the S and G2 phase, as the process needs factors expressed in those parts of the cell cycle. The new sequence can be copied and passed on through dividing cells too (10). In another design, the CRISPR-Cas technology will just cut the genome, a process called Non-Homologous End Joining (NHEJ). Without a template DNA, after the genome is cut, random base pairs may be inserted or deleted in joining the two ends. Ultimately, this messes up the DNA sequence, which usually introduces mutations, reading frame shift, and premature stop codons, and is a troublesome occurrence (11). Another result of cutting DNA without a template is Microhomology-Mediated End Joining, or MMEJ. In MMEJ, the cell looks for short strands of DNA to insert into the new cut, which often results in large deletions (12). Scientists take advantage of the latter two designs to generate mutations in genomes of model organisms to determine functions of the affected genes or regulatory elements.

However, NHEJ is vital in gene knockout, or disabling a specific gene. In gene knockout, CRISPR will cut the genome, and indels (deletions or insertions) occur to rejoin the DNA. Then, the DNA is cut again, more base pairs are inserted or deleted, until CRISPR no longer recognizes that area and stops cutting the DNA. As such, the targeted gene has been disabled.

HISTORY OF CRISPR

First discovered by Dr. Yoshizumi Ishino and his team in 1987 in bacteria, CRISPR's purpose puzzled scientists (13). However, in 1995, Dr. Francisco Mojica of the University of Alicante discovered a structure like CRISPR in the archaeal genome of *Haloferax*

mediterranei, which led scientists to believe that, if CRISPR is showing up in two different systems, it must have some greater use. After further research, Mojica hypothesized that CRISPR was an immune system (3). Experiments on how CRISPR worked as an immune system were conducted, but it was not until 2012, when Jennifer Doudna and Emmanuelle Charpentier published a paper on cloning Cas9 (a critical DNA-cutter enzyme of the CRISPR-Cas system), that people realized that this CRISPR-Cas system could be adopted as a greatly useful gene-editing tool. Drs. Doudna and Charpentier won the Nobel Prize in Chemistry in 2020 for this discovery (14).

Later, Drs. George Church's and Feng Zhang's groups published the first two papers to utilize the CRISPR-Cas system for eukaryotic genome editing (6, 15). Soon afterwards, publications of CRISPR-Cas technology boomed, and scientists since have been excited to research how they could use this DNA-cutting technology for eukaryotic cells and humans.

Conducting genome editing in eukaryotic cells has been a long-time dream of scientists. In the 1980s and 1990s, Drs. Mario Capecchi, Oliver Smithies, and Sir Martin Evans discovered that in eukaryotic cells, a very low level of homologous recombination occurs when DNA is spontaneously broken, and with that, cells might incorporate an extraneous copy of DNA into their genome (16). Dr. Capecchi, Smithies, and Evans won the 2007 Nobel Prize in Physiology and Medicine for their ground-breaking work on gene targeting using embryonic stem (ES) cells (17). Nevertheless, the chances of this kind of DNA integration are very low even in ES cells, and the targeting DNA could also be incorporated in areas where it was not supposed to. Thus, scientists have been thinking about cutting DNA to increase the chance of HDR, but cutting the DNA at the right spot was also a big challenge. Researchers have previously used homing endonucleases, but to cut DNA at different spots, they would need different enzymes. This prompted research groups to focus on target sites.

One such strategy was to use rare cutting endonuclease enzymes to create specific double strand breaks. However, due to target sites being too rare, and the possibility of the cut causing Non-Homologous End Joining (NHEJ), where nucleotides may be wrongly inserted or deleted, this strategy was abandoned (11). Even after scientists repurposed meganucleases (DNA scissors) to cut DNA, only certain genomes could be targeted. Then, researchers looked towards eukaryotic Zinc-Finger Nucleases (ZFN).

ZFN were first introduced in the early 2000s (18). Scientists engineered a fusion protein to combine non-specific DNA-cutting enzymes—cuts DNA but does not know exactly where—with DNA binding proteins to recognize the DNA sequences and location. That way, DNA can be recognized and cut. Later, in 2010-2011, Transcription Activator-Like Effector Nucleases, or TALENs, were proposed as a genome editing tool (19). Like ZFN, they offer a way to locate a specific area of the DNA sequence to cut. However, both ZFN and TALENs use proteins to recognize DNAs. In contrast, CRISPR uses RNA to locate and bind to parts of the genome, which is more specific, scalable, and efficient. Therefore, after the 2012 publication on Cas9, scientists focused on CRISPR as the main technology for gene editing (1).

BREAKTHROUGHTS & CLINICAL TRIALS

Since its discovery in 1987 up to 2025, CRISPR-Cas technology has had many breakthroughs, and there are more than 270 currently ongoing clinical trials.

BREAKTHROUGHS

In these past years, CRISPR-Cas technology has made huge advancements. Briefly, the research frontlines focus on how to make this technology safer (less off-target effects with AI tools), more efficient and be able to cover the whole genome (develop new gRNAs and discover new Cas proteins), work in non-dividing cell types (neurons and others), and personalized. Below list just a few detailed examples of them:

Personalized Gene Editing

This year, scientists have had a breakthrough with personalized CRISPR-Cas treatment (20). In a short span of six months, researchers developed a CRISPR-Cas therapy treatment for an infant named KJ, who had a rare metabolic disease called severe carbamoyl phosphate synthetase 1 (CPS_1) deficiency (found only in 1 of 1.3 million newborns) (21). This mutation is expressed in creating a lack of enzymes to process nitrogen into urea, and without this enzyme, nitrogen turns into toxic ammonia in the bloodstream, which is damaging to the brain. Patients with this disease will typically need a liver transplant, but there are many complications that come along with that, making it a less-than-ideal option. Since his birth, KJ has had to remain in Children's Hospital of Philadelphia under

close watch.

Researchers Ahrens-Nicklas and Kiran Musunuru, who have worked towards creating customized gene editing treatments since 2023, took on this challenge. Within a short span of six months, they were able to create base-editing therapy to correct KJ's mutation. KJ received his first dose in February of 2025, then two more in March and April respectively. There are no reports of serious side effects, and after receiving this treatment, KJ has been discharged from the hospital (20).

Neuron Repair

Researchers at Stanford, Professor Stanley Qi and his lab, have discovered a way to repair neurons using the CRISPR-Cas technology (22). Essentially, the CRISPR-Cas technology (specifically CRISPR-Cas13) was first mutated, losing the DNase activity and then adding a subcellular localization signal. These modifications allow the mutated protein to act as an RNA mailman: it carries mRNA molecules (which produce proteins) to promote neuronal growth to a specific subcellular location of neuron, the neurites. This new technology is being called spatial RNA medicine, and the re-engineered CRISPR-Cas13 is now named CRISPR-TO. Combined with different localization signals, the CRIPR-TO can bring RNAs to different parts of the neurons.

Using spatial 'zip codes,' CRISPR-TO is able to transport RNAs and thus proteins to where they are mostly needed. Previously, without this technology, neurological disorders may prevent the RNA from getting where it needs to go. Not only can CRISPR-TO get around this, but it may be more effective as well: during one screening process, one RNA increased neuron growth by 50% in just 24 hours. Offering more precision and control over RNA medicine, CRISPR-TO has opened a new door for neurodegenerative diseases.

TIGR System

TIGR, otherwise known as Tandem Interspaced Guide RNA, is an ancient system that uses RNA to guide them to specific parts of the DNA. Adopting TIGR could simplify the CRISPR system immensely.

Compared to the guide RNA for systems like CRISPR, TIGR's guide RNA is much more compact (23). This makes the delivery part of gene editing easier. But most importantly, whereas the CRISPR-Cas technology's guide system, as mentioned earlier, needs a short DNA sequence called PAMs, TIGR's does not and can be programmed to bind to any area of interest. Essentially, whereas the CRISPR-Cas technology can only edit the DNA that's marked by PAMs, TIGR can edit the genome anywhere. If this technology can be properly incorporated into CRISPR-Cas technology, it can allow scientists to target and treat diseases beyond present limitations.

AI in CRISPR-Cas Technology

The integration of AI into the CRISPR-Cas technology can make the process much safer and more efficient. With machine learning, the identification of targets can become more precise, and predictions for side effects (both on and off target) become much more accurate, reducing accidents and making the whole approach that much safer (24). Additionally, AI can help to discover more CRISPR related proteins, opening new doors for gene therapy. Both CRISPR and AI have proven themselves to be powerful tools. Their combination will have limitless possibilities.

An example of this is Guide-Guard, an AI tool introduced in 2022 which uses CRISPR's gRNA to predict off-target risks, therefore protecting against off-target sites. Guide-Guard has been found to have 84% accuracy (25). More recently, a model called TIGER has been invented. TIGER is an AI model trained by a dataset of gRNAs to predict the efficacy of the gRNA. This increases the safety of the CRISPR-Cas technology as well (26).

As seen, the CRISPR-Cas technology is rapidly advancing. The personalized gene therapy will allow it to target and treat rare, unstudied genetic-mutation caused diseases, the neuron repair creates a new use for the technology in non-dividing cells, and TIGR and AI will help the CRISPR-Cas technology to do its job safer and more efficient. With this progress, the technology evolves into an even more hopeful and powerful treatment for diseases.

CRISPR-RELATED CLINICAL TRIALS

Currently, there are many ongoing CRISPR-related clinical trials for treating different diseases. The following charts (Figures 1 and 2) and graph (Figure 3) summarize the major clinical trials conducted to date, outlining their objectives, study phases, and overall progress up to February 2025 (27).

Figure 1 describes the number of clinical trials in 16 different therapeutic areas, including cancers, disorders, deficiencies, and others. Cancers currently have the greatest number of clinical trials, with blood cancers

at 28.8% and solid cancers at 17.2%. Bacterial diseases (4 clinical trials) and hemophilia (1 clinical trial) are unlabeled due to the sparse frequency. Neurological conditions (0 clinical trials) are unmentioned. Figure 2 illustrates the development stages (Phase I, II, or III) of clinical trials. A majority of the trials are in Phase I, a little over 70%. Figure 3 describes the stages of clinical trials by therapeutic areas. For all therapeutic areas, the most clinical trials are still in Phase I. This is not surprising, given that CRISPR is such a new technology, so clinical trials are fairly new.

In summary, as we can see from the graphs, CRISPR technology is most frequently used in the areas of blood related disease and cancers, such as blood cancers, haemoglobinopathies, and solid cancers. This is likely because these diseases are mostly driven

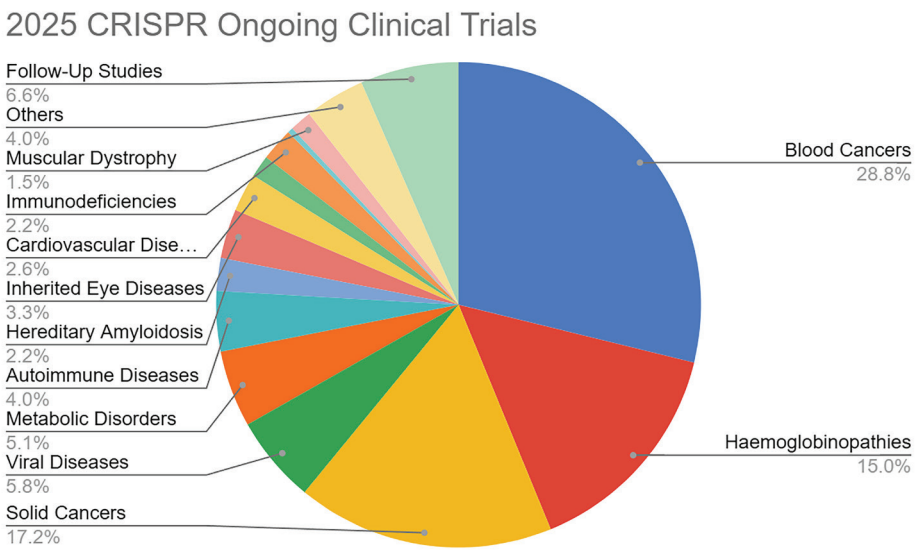

Figure 1. A pie chart illustrating the percentage of CRISPR clinical trials across a broad range of diseases.

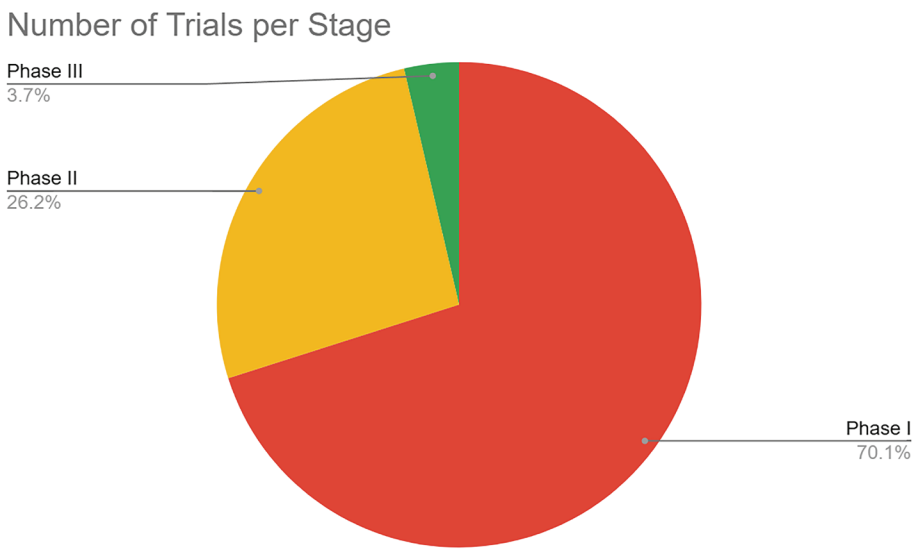

Figure 2. A pie chart illustrating the percentage of clinical trials in each stage (Phase I, Phase II, or Phase III) across the top 16 therapeutic areas.

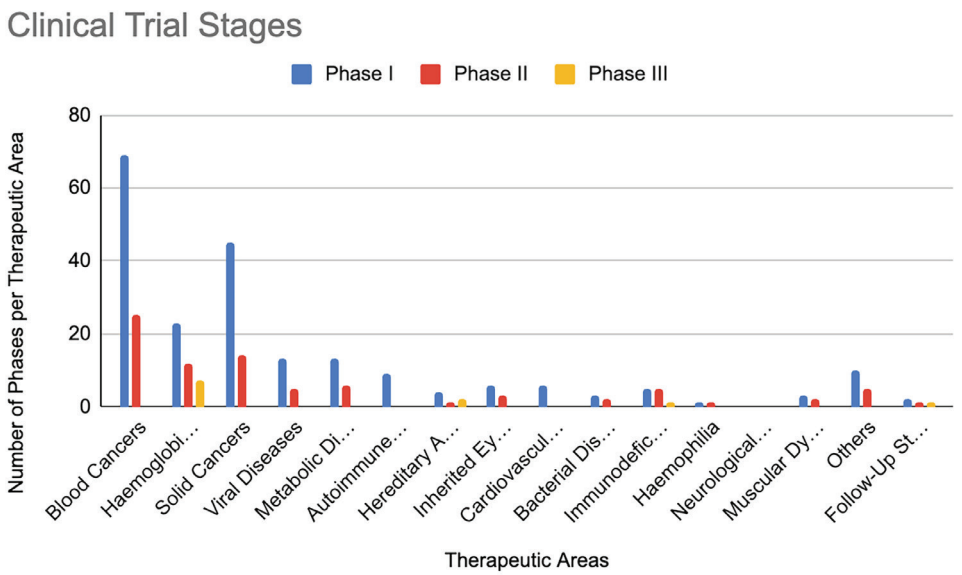

Figure 3. A column chart illustrating the stages of clinical trials in the top 16 therapeutic areas. The blue bar represents number of clinical trials in the Phase I, red bar for the Phase II, and yellow bar for the Phase III.

by a single gene mutation and the affected cells are renewable, which makes applying the CRISPR-Cas technology more feasible. In contrast, at present there are no ongoing clinical trials of neurological conditions, possibly because of the number of genes involved and multisource of disease pathology and few neurons can't be regenerated *in vivo* so far.

Additionally, most of the clinical trials are still in Phase I, reflecting that this technology is still very new, although with great potential. However, a few of the trials have advanced to Phase II, and even Phase III. For example, the Phase III clinical trial NCT05329649 is targeting at Sickle Cell Disease among pediatric participants with severe SCD and hydroxyurea failure or intolerance. With anticipation, the breakthrough of a new treatment will likely come from those areas first.

ETHICAL CONSIDERATIONS IN GENOME EDITING

Because the CRISPR-Cas technology enables targeted modifications to the human genome, its potential misuse in germline editing raises significant ethical concerns. When scientist Jiankui He modified human embryos for HIV resistance in 2018, it resulted in the birth of two twin girls (28). The practice violated principles such as the Nuremberg Code, which set up principles for medical experiments on human subjects (29). This gained worldwide attention and spurred conversation on the ethics of gene editing.

While somatic editing (editing non-reproductive cells) for treating disease is typically supported, germline genetic editing (involving sperm, eggs, or embryos) has great ethical concerns. At present, the World Health Organization (WHO) guidelines list informed consent as one of the requirements of gene editing (30). The US National Academies of Science, Engineering, and Medicine specified that germline research should only be allowed if there are extreme circumstances and there are no other reasonable options left (31). Although germline genetic editing may be beneficial for solving health problems and correcting genetic mutations at the root, there are still overweighing problems at the current stage. The effects of the CRISPR-Cas technology is difficult to reverse, raising issues such as safety due to current limited knowledge about functions of the targeted gene, off-target editing, and passing those unintentional genetic mutations onto offspring (28). Thus, germline editing is currently prohibited by most countries including the United States (32, 33).

Additionally, germline editing can be a slippery slope to designer babies. It is possible for people to undergo gene editing in pursuit of "preferred" traits such as strength, speed, or even appearance features like eye color (34). Many ethical concerns associated with these potential practices. Does this alter human nature? Will it be a privilege that only the rich will

have access to? Or will this eventually exclude those considered biologically inferior?

As CRISPR-Cas technology continues to grow more powerful, these debates and discussions will increase too. It will take international collaboration, human society consensus, and appropriate regulatory oversights to keep CRISPR-Cas technology practice in the most ethical way.

CONCLUSION

Overall, CRISPR-Cas technology is an extremely powerful tool for the biomedical field. In about 30 years, this technology has rapidly evolved to become more efficient and safer, making treatment for both genetic and rare diseases so much easier and more feasible. With over 270 ongoing clinical trials, constant new breakthroughs, the incorporation of artificial intelligence, and personalized treatment option, CRISPR-Cas technology is a hopeful promise for rare and genetic diseases. Nevertheless, it is extremely important to find a balance between technology advancements and human ethics. In the future, the CRISPR-Cas technology will be used to modify genomes of more species, such as the mosquito, for blocking transmission of infectious diseases, to modify grains and plants for producing more food, or even to modify plants so they can survive on other planets, such as Mars. It is exciting and inspiring to imagine what impact this technology will have on humanity down the road.

CONFLICT OF INTERESTS

The author declares no conflicts of interest related to this work.

REFERENCES

1. Pacesa, M., O. Pelea, and M. Jinek, Past, present, and future of CRISPR genome editing technologies. *Cell*. 2024; 187 (5): 1076-1100. https://doi.org/10.1016/j.cell.2024.01.042
2. Wang JY and Doudna JA. CRISPR technology: A decade of genome editing is only the beginning. *Science*. 2023; 379 (6629): eadd8643. https://doi.org/10.1126/science.add8643
3. Mojica FJ, *et al*. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol*. 2005; 60 (2): 174-82. https://doi.org/10.1007/s00239-004-0046-3
4. Barrangou R, *et al*. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*. 2007; 315 (5819): 1709-12. https://doi.org/10.1126/science.1138140
5. Knott GJ and Doudna JA. CRISPR-Cas guides the future of genetic engineering. *Science*. 2018; 361 (6405): 866-869. https://doi.org/10.1126/science.aat5011
6. Cong L, *et al*. Multiplex genome engineering using CRISPR/Cas systems. *Science.* 2013; 339 (6121): 819-23. https://doi.org/10.1126/science.1231143
7. Yu SY, *et al*. Increasing the Targeting Scope of CRISPR Base Editing System Beyond NGG. *CRISPR J.* 2022; 5 (2): 187-202. https://doi.org/10.1089/crispr.2021.0109
8. Karvelis T, Gasiunas G, and Siksnys V. Methods for decoding Cas9 protospacer adjacent motif (PAM) sequences: A brief overview. *Methods*. 2017; 121-122: 3-8. https://doi.org/10.1016/j.ymeth.2017.03.006
9. Jasin M and Rothstein R. Repair of strand breaks by homologous recombination. *Cold Spring Harb Perspect Biol.* 2013; 5 (11): a012740. https://doi.org/10.1101/cshperspect.a012740
10. Lin S, *et al*. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. *Elife.* 2014; 3: e04766. https://doi.org/10.7554/eLife.04766
11. Pawelczak KS, *et al*. Modulating DNA Repair Pathways to Improve Precision Genome Engineering. *ACS Chem Biol*. 2018; 13 (2): 389-396. https://doi.org/10.1021/acschembio.7b00777
12. Sfeir A, Tijsterman M, and McVey M. Microhomology-Mediated End-Joining Chronicles: Tracing the Evolutionary Footprints of Genome Protection. *Annu Rev Cell Dev Biol*. 2024; 40 (1): 195-218. https://doi.org/10.1146/annurev-cellbio-111822-014426
13. Ishino Y, *et al*. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *J Bacteriol.* 1987; 169 (12): 5429-33. https://doi.org/10.1128/jb.169.12.5429-5433.1987
14. The Nobel Prize in Chemistry 2020. Available from: https://www.nobelprize.org/prizes/chemistry/2020/press-release/ (accessed on 2025-08-15).
15. Mali P, *et al*. RNA-guided human genome engineering via Cas9. *Science.* 2013; 339 (6121): 823-6. https://doi.org/10.1126/science.1232033
16. Capecchi MR. Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. *Nat Rev Genet*. 2005; 6 (6): 507-12. https://doi.org/10.1038/nrg1619
17. The Nobel Prize in Physiology or Medicine 2007. Available from: https://www.nobelprize.org/prizes/medicine/2007/7598-the-nobel-prize-in-physiology-

or-medicine-2007-2007/ (accessed on 2025-08-15).
18. Jo YI, Kim H, and Ramakrishna S. Recent developments and clinical studies utilizing engineered zinc finger nuclease technology. *Cell Mol Life Sci*. 2015; 72 (20): 3819-30. https://doi.org/10.1007/s00018-015-1956-5
19. Joung JK and Sander JD. TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol*. 2013; 14 (1): 49-55. https://doi.org/10.1038/nrm3486
20. World's First Patient Treated with Personalized CRISPR Gene Editing Therapy at Children's Hospital of Philadelphia. Available from: https://www.chop.edu/news/worlds-first-patient-treated-personalized-crispr-gene-editing-therapy-childrens-hospital (accessed on 2025-08-15).
21. Carbamoyl Phosphate Synthetase I Deficiency. Available from: https://medlineplus.gov/genetics/condition/carbamoyl-phosphate-synthetase-i-deficiency/ (accessed on 2025-08-15).
22. New CRISPR technology could help repair damaged neurons. 2025; Available from: https://biox.stanford.edu/highlight/new-crispr-technology-could-help-repair-damaged-neurons (accessed on 2025-08-15).
23. The New TIGR-Tas Gene Editing System. Available from: https://theness.com/neurologicablog/the-new-tigr-tas-gene-editing-system/ (accessed on 2025-08-15).
24. Revolutionizing CRISPR technology with artificial intelligence. Available from: https://www.nature.com/articles/s12276-025-01462-9 (accessed on 2025-08-15).
25. Bingham J, Arussy N, and Zonouz S. Guide-Guard: Off-Target Predicting in CRISPR Applications. 2022. *Cham: Springer International Publishing.* https://doi.org/10.1007/978-3-031-21753-1_41
26. Wessels H-H, *et al*. Prediction of on-target and off-target activity of CRISPR-Cas13d guide RNAs using deep learning. *Nature Biotechnology*. 2024; 42 (4): 628-637. https://doi.org/10.1038/s41587-023-01830-8
27. Gene-editing Clinical Trials. Available from: https://crisprmedicinenews.com/clinical-trials/ (accessed on 2025-08-15).
28. Rothschild J. Ethical considerations of gene editing and genetic selection. *J Gen Fam Med*. 2020; 21 (3): 37-47. https://doi.org/10.1002/jgf2.321
29. Nuremberg Code: Directives for Human Experimentation. Available from: https://ori.hhs.gov/content/chapter-3-The-Protection-of-Human-Subjects-nuremberg-code-directives-human-experimentation (accessed on 2025-09-15).
30. WHO releases new principles for ethical human genomic data collection and sharing. Available from: https://www.who.int/news/item/20-11-2024-who-releases-new-principles-for-ethical-human-genomic-data-collection-and-sharing (accessed on 2025-09-14).
31. Ethical considerations for human gene editing. Available from: https://www.nationalacademies.org/our-work/human-gene-editing-scientific-medical-and-ethical-considerations (accessed on 2025-09-25).
32. Brokowski C and Adli M. CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool. *J Mol Biol*. 2019; 431 (1): 88-101. https://doi.org/10.1016/j.jmb.2018.05.044
33. Germline gene editing is prohibited in the United States. Available from: https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/united-states-embryonic-germline-gene-editing/ (accessed on 2025-09-25).
34. Ranpara M. Designer babies. *Can Med Educ J*. 2020; 11 (1): e147-e148. https://doi.org/10.36834/cmej.68361