REVIEW ARTICLE

CRISPR in Medicine: A Review of the Technology, Challenges, and Applications in Clinical Trials

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Abstract

CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats—Cas9 protein associated) is a breakthrough technology that can be easily programmed for a precise gene editing by modifying existing genes or introducing new ones via the cell's double-strand break repair pathway (DSB). Many genetic diseases were treated with this technology, including sickle cell anemia, beta thalassemia, leber congenital amaurosis (LCA), leukemia, lymphoma, and lung cancer. Several clinical trials were conducted; some resulted in success, while others identified areas of improvement. An appropriate application of technology can provide significant improvements in biological research and treatment of previously difficult-to-treat diseases, such as cancer, HIV, and sickle cell anemia. Cas9, which is the most extensively used gene editing nuclease, has a significant promise for the treatment of cancer, viral infections, and genetic disorders among other conditions. Recent research also identified additional CRISPR-Cas system varieties, including superFi CRISPR or miniCas, which can be applied for gene editing. Despite the quick advancements in basic research and clinical trials, numerous fundamental issues, such as editing effectiveness, relative delivery difficulties, off-target consequences, and immunogenicity, present major ongoing obstacles. Therefore, this review highlights the recent applications and challenges in the CRISPR-Cas9 technology in clinical trials.

Keywords

Clinical trials; CRISPR-Cas9; Gene editing technology; Genetics; Mutant DNA

Introduction

With the gene as the basic unit of heredity,[1] the ability to make specific modifications in a genome is essential to combat diseases. One of the most promising techniques for accomplishing this goal is gene therapy, which has the potential to cure cancers, infections, and genetic disorders. The human genome contains ~25,000 genes;[2] therefore, any gene mutations can cause genetic disorders. For instance, an HTT gene mutation can cause Huntington's disease.[3] Mutations in tumor suppressor genes can cause cancers; for example, a mutation in HER2 proto-oncogene can trigger breast cancer.[3,4] Mutations can also occur ubiquitously and at a higher rate due to environmental factors, such as radiation or UV light exposure.[5] These mutations could also be inherited through many generations.[6] With the completion of the human genome project, genetics has become a major focus of research in clinical medicine. Gene therapy,[7] where a genome is modified to treat or cure a disease, is one method to combat these diseases. This can be accomplished by restoring mutated genes *in vivo* or *ex vivo*. Recombinant DNA technology, in which normal DNA fragments are generated in a lab setting and put into a genome via a vector, is a common strategy.[8,9] These vectors often pose large challenges, as they must be efficient in releasing one or more genes depending on the size, without invoking an immune response.[10] The most used vectors can be plasmodial, nanostructured, or viral.[11] This is because of their efficacy in invading cells. For instance, when the infectious part of a virus is removed, viruses can accurately and safely deliver DNA fragments to the nucleus of cells. However, using viral vectors as the primary means of expressing gene therapy is ineffective due

to the significant risk of off-target effects and adverse immune responses.[12] For instance, in its first experiment in 1999, the most widely publicized case was that of a 17-year-old Jesse Gelsinger who died while receiving a potential treatment for ornithine transcarbamylase (OTC) deficiency.[13-15] This death was ascribed to uncertainties in gene therapy, so the field took a significant step back for clinical applications. There are currently relatively few FDA-approved treatments that use this technology, and they are all primarily ex vivo, making it exceedingly exclusive and expensive. For instance, a single therapeutic dose of YESCARTA, a gene therapy medication that targets B-cell lymphoma, costs $373,000 USD.[16]

Gene editing

Gene editing relies around the natural cellular process of double-strand break repair (DSBR), by which cells repair their DNA after breakage.[17] Genome editing re-emerged in 2012 with the development of CRISPR/Cas9, a genetic editing tool derived from a primordial cellular system of certain bacteria against a phage, a virus capable of infecting bacteria. This technique was used to successfully evaluate a wide range of experimental models, including cell lines, laboratory animals, plants, and human clinical trials. In the CRISPR/Cas9 system, the Cas9 nuclease is programmed to generate a site-directed double-stranded DNA break using a short RNA molecule as a guide.[18] Using a method that permanently alters the genomic target sequence, DNA damage can be repaired. These DNA alterations can occur internally (during DNA replication in meiosis) and externally (due to radiation and specific chemotherapeutic drugs). Other researchers who examined yeast cells using *Saccharomyces cerevisiae* demonstrated that improperly performed DSBR can lead to genetic changes, gene deletions, chromosomal abnormalities, and even cell death. Homology-directed repair (HDR) and non-homologous end joining (NHEJ) are the mechanisms by which eukaryotic cells typically perform DSBR.[19] Few base pairs of homology are required for NHEJ between two broken DNA ends. Since NHEJ occurs without a homologous DNA template, the repair and its characterization are susceptible to errors, resulting in unintended mutations. Additionally, this type of repair can result in unintended genetic deletions. This can happen at any cell cycle stage and is the typical DSBR mechanism for cells. In contrast, homologous recombination is a more accurate method of repair that requires the use of a homologous repair template. Compared to NHEJ, HDR often occurs in the late S or G2 phase, with a sister chromatid as the repair template. The process involves end resection, strand invasion, DNA synthesis, Holliday junctions, ligation, and precise repair. HDR is rarely conducted by cells because NHEJ is a faster process that either rejoins split ends or uses conveniently accessible DNA to replace the lost DNA. Given that cells can execute precise repairs, safe gene editing technologies seek to activate HDR as the DSBR process by providing cells with a new homologous DNA template rather than a conventional sister chromatid template. Figure 1 shows a schematic illustration of HDR and NHEJ.

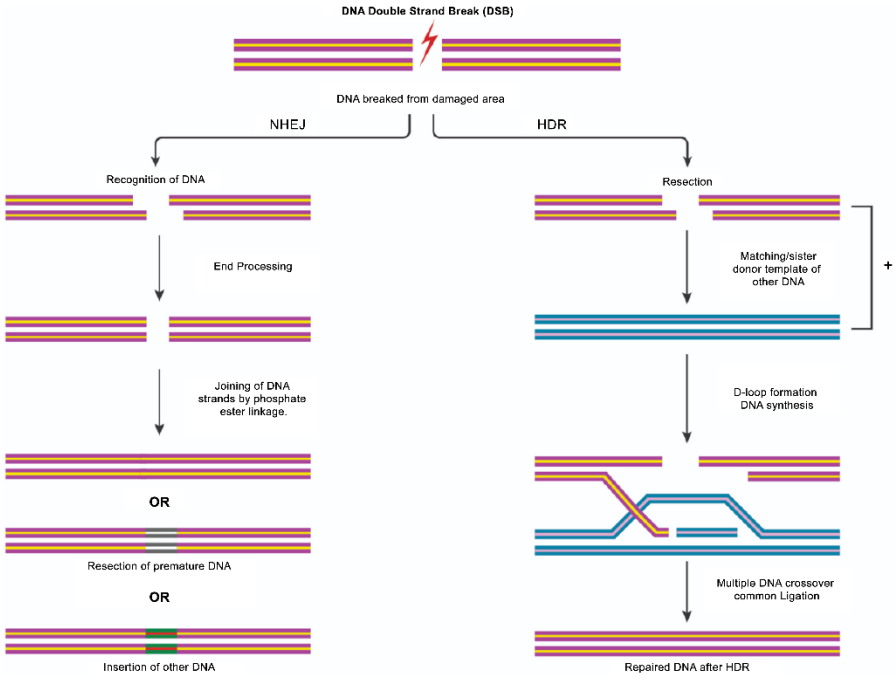

Figure 1. Comparison of HDR and NHEJ mechanisms. HDR, homology-directed repair; NHEJ, non-homologous end joining.

NHEJ is a natural genetic response to a deleted gene. HDR is exploited for gene correction and addition. Typically, gene editing technologies utilize HDR for repairs and additions. A single- or double-stranded exogenous DNA template is required for this process and is commonly introduced using gene editing tools, such as the template strand delivered by CRISPR Cas-9.

CRISPR Cas-9

Scientists utilize CRISPR Cas-9 to generate specific breaks in the genome of an organism. CRISPR–Cas9 has revolutionized biological sciences by enabling the precise modification of a genomic material. As a result of targeted DNA breaks, cells utilize their intrinsic DNA repair mechanisms to bring about the intended changes. Random modifications like indels or point mutations are introduced by imprecise double-strand repair, whereas precise editing restores or selectively edits the locus, as prescribed by an endogenous or exogenous template. The natural cellular DNA repair pathway, HDR, is employed by substituting a customized DNA template. As shown in Figure 2, a Cas-9 complex constitutes the Cas9 protein and guide RNA, with the protein constituting six domains: REC I, REC II, Bridge Helix, PAM Interacting, HNH, and RuvC.[20]

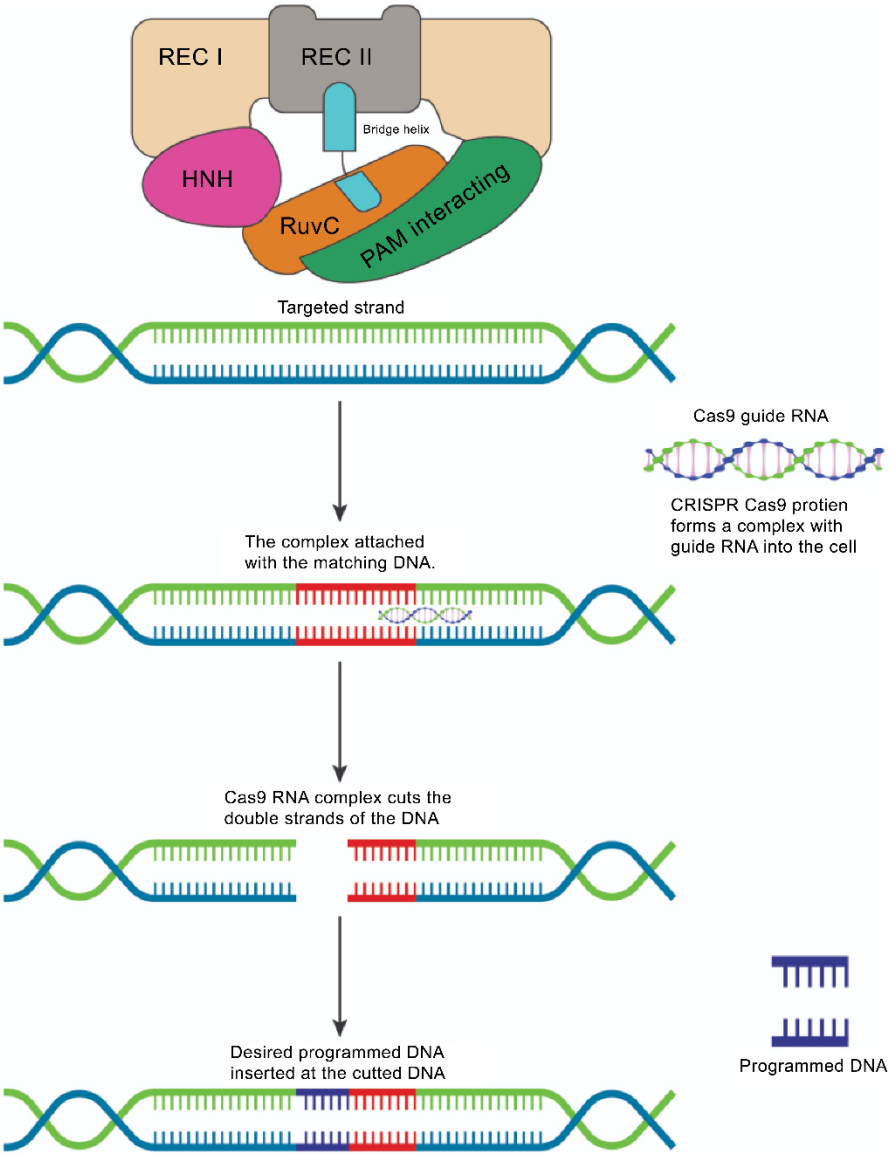

Figure 2. Schematic of the CRISPR Cas-9 system. REC I's primary function is to bind the guide RNA to the complex and structural properties. The role of the REC II domain is not yet well-understood. The bridge helix is rich in arginine and is essential in initiating the cut. The PAM interacting aspect is responsible for binding to the target DNA. HNH and RuvC domains make the actual cut.

Ethical dilemmas

The modification of genomes of gametes (eggs and sperm) and early embryos is known as germline editing. These modifications to humans have an effect on both the individual and their offspring. Theoretically, they may enhance beneficial traits and treat sickness. In October 2018, China's "CRISPR babies" were named after Chinese scientist He Jianku, who modified the embryos of two twins to give them resistance to HIV infection. Although this experiment was intended to investigate the potential of gene editing, it was controversial.[21] In the study, Jianku disregarded scientific and ethical norms, sparking criticisms in the bioethics community.

At the 2015 International Summit on Human Gene Editing meeting in Napa Valley, ethicists and CRISPR-Cas9 developers explored the ethical, legal, and biomedical aspects of CRISPR systems.[22] In February 2017, a multidisciplinary NASEM committee published a detailed study on human genome editing. The Committee favored somatic genome editing but did not permit genomic modification for any enhancement. NASEM states, "*In light of the technical and social concerns involved… heritable genome-editing trials might be permitted, but only following much more research to meet existing risk/benefit standards for authorizing clinical trials and even then, it was only for compelling reasons and under strict oversight…*" As it stands, the United States federal law does not permit funding for research involving human embryos; however, a NASEM report proposes that clinical trials incorporating germline editing are permissible following a detailed review of technical and safety risks.[23]

Several ethical considerations, such as safety, informed consent, equity, and value for human embryos, restrict the advancement of this technology. Given the costly history of gene therapies, there is no question that ex vivo gene editing techniques will also be expensive and time-consuming. Considering that gene therapies utilizing TALENs and Zinc-finger nucleases provide cures for genetic diseases that often have no alternative therapeutic options, the price of any official treatments will be exorbitantly expensive. Zolgensma, a one-time virally delivered gene therapy intended to provide a fully functional copy of the defective SMN1 gene that causes spinal muscular atrophy, costs $2 million per treatment. Even though the cost of manufacturing is only a few dollars, gene therapies must account for the cost of research, development, and clinical trials, necessitating a high price point so pharmaceutical companies will earn a profit.[24-26]

In addition, ex vivo treatments involve lengthy procedures in which cells must be extracted, genetically modified, and administered; this can take up to one month for certain therapies. Despite its potential life-saving benefits, CRISPR treatments are neither feasible nor accessible to the general public due to these obstacles. They violate a fundamental bioethical justice standard: the treatment cannot be administered equitably. Still, this is merely an obstacle that gene editing must overcome. One of the primary reasons for such a high price is that the technology has only recently emerged from clinical trials. In accordance with bioethical standards, the costs of such treatments will probably decrease significantly over time and as a result of research and application advancements for the general public.[26]

Bioethical challenges resulting from germline genome editing can be understood from two perspectives: its failure and success. In the case of failure, the notion that genome modifications can be passed on to future generations is a major concern. The risk-benefit ratio of informed consent as an ethical guideline is often deemed less significant than the consequences of a faulty genome editing. The possibility of creating numerous mutations and adverse effects can result in the transmission of unwanted alterations to future generations. Further, scientists discovered that CRISPR-Cas9 mutations are very common and must be improved upon for the future.[27] These non-target mutations exclusively occur in exon areas; hence, the number of mutations is far higher than anticipated. However, recently discovered CRISPR technologies aim to counteract these difficulties. Another issue arises in genetic mosaicism – the presence of genetically different somatic cell populations in an organism and is often masked. Mosaicism can lead to major phenotypic changes and formation of fatal genetic mutations.[24,28,29] Thus, nuclease cleavage sites should be confirmed to eliminate the possibility of mosaicism. The limited access to clinical research on embryos makes the consequences unknown for gene editing germline cells. Otieno (2015) claims that "the possible side effects cannot be predicted before birth, and the consequences are unknown."

If germline genome editing is successfully applied, the fundamental issue of non-therapeutic uses of the technology arises. From a scientific standpoint, the potential of breeding and manipulating an embryo to eliminate all genetic problems and create "perfect" babies could be investigated. However, the core notions of natural selection may be essentially abolished, raising fresh issues about the human species' place in the universe. It is possible that skin color and other traits will be modified in the future. The fate of a child is another bioethical dilemma of an effective treatment. In the case of unfavorable side effects, an informed

consent is important because the child has no opinion or choice in the process. On the other hand, a clear informed consent can be granted for genome-edited somatic cells, making germline editing more difficult.

Technological limitations

Although CRISPR/Cas9 is a powerful technology, it has significant drawbacks.

- For many therapeutic applications, it is still difficult to deliver CRISPR/Cas materials to mature cells in significant quantities. The most frequent form of delivery is by viral vectors.
- Cells that take up CRISPR/Cas may not produce the desired modifications since genetic modifications are not guaranteed.
- "Off-target" modifications, albeit uncommon, may have serious repercussions, especially in clinical applications.

Furthermore, pre-existing antibodies against Cas9 are a major concern, as evidenced by a human study that revealed more than 50% immunity against bacterial nucleases. Moreover, gRNA causes an innate immune response in human cells. These concerns are mostly for in vivo gene therapy; as a result, ex vivo options for future CRISPR technologies are being explored.[30,31]

Discussion

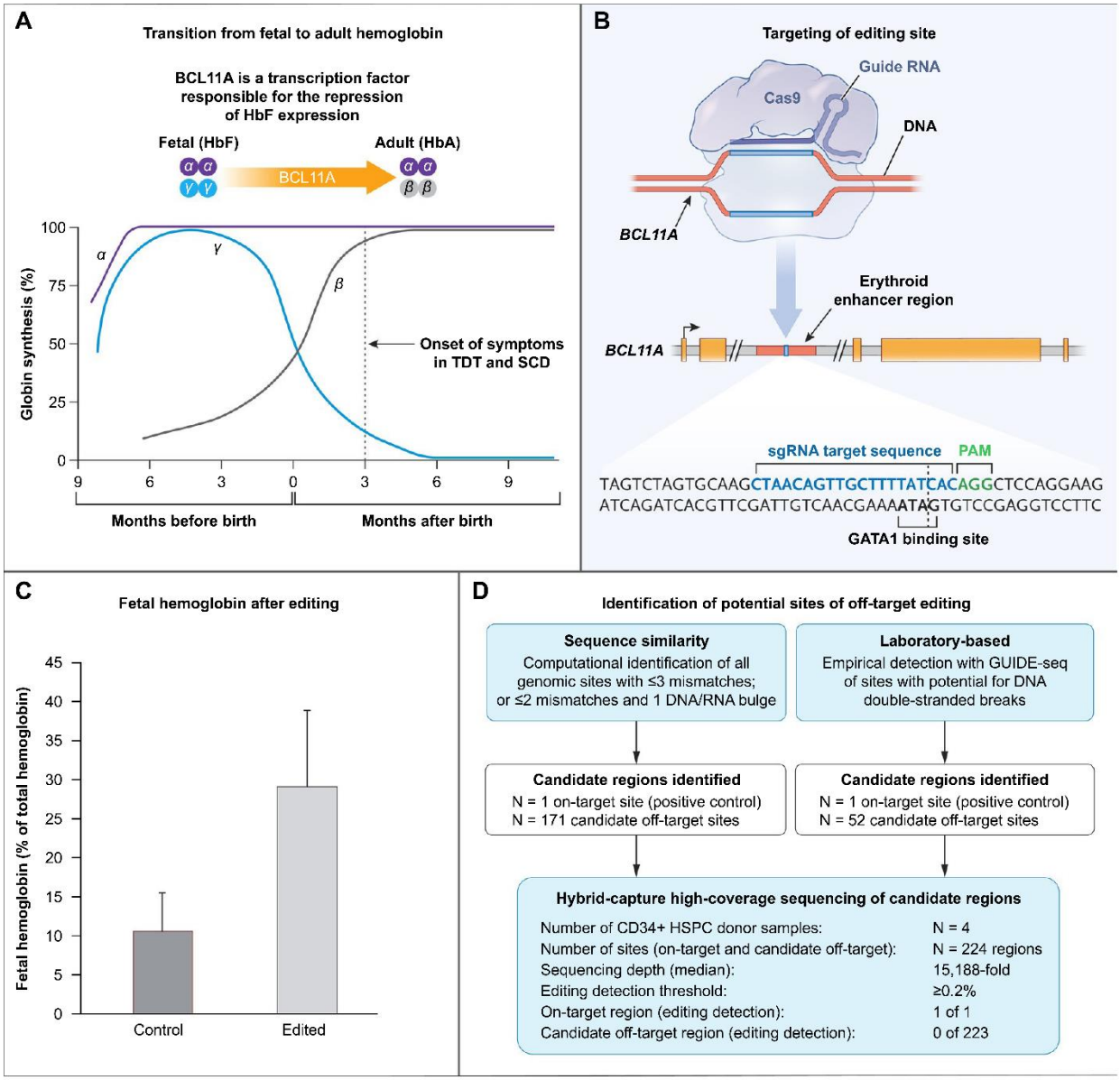

Figure 3. A. After a person is born, there is a transition from fetal hemoglobin (HbF) to adult hemoglobin (HbA). The transcription factor BCL11A plays a role in this process by suppressing the production of γ-globin, a component of fetal hemoglobin. In individuals with sickle cell disease (SCD) or transfusion-dependent β-thalassemia (TDT), an inability to produce functional or sufficient amounts of β-globin leads to the onset of symptoms around 3 months after birth, when fetal hemoglobin levels decrease. B. The single guide RNA (sgRNA) directs the CRISPR-Cas9 complex to a specific location in the erythroid-specific enhancer region of the BCL11A gene. This location, known as the target editing site, is depicted as gold boxes representing the five exons of the BCL11A gene. The GATA1 transcription facts binds to the site marked as “GATA1,” and the protospacer adjacent motif (PAM) is a specific DNA sequence (NGG) that must be present immediately following the Cas9 target DNA sequence. C. This data represents the percentage of fetal hemoglobin in relation to total hemoglobin in samples taken from 10 healthy donors, after

editing and differentiation of erythroid cells. The error bars represent standard deviation and data is collected from preclinical studies. D. This figure presents the results of an evaluation of off-target effects. The methods used were genome wide unbiased identification of double-strand breaks enabled by sequencing (GUIDE-seq) and hybrid capture o hematopoietic stem and progenitor cells (HSPC). GUIDE-seq was performed independently on 3 samples of CD34+ HSPC from healthy donors, and hybrid capture was then performed on 4 additional samples of CD34+ HSPC from healthy donors to confirm the nominated sites. On-target allelic editing was confirmed in each experiment at an average of 57%, with no detectable off-target editing observed at any of the sites identified by GUIDE-seq or by sequence homology.

Figure 3 shows the switch that naturally occurs in humans from fetal hemoglobin (HbF) (only used by fetuses) to adult hemoglobin (HbA), with the reason for the swap being unknown. HbF is not affected by sickle cell mutations, so replacing HbA with HbF is a treatment option for sickle cell disease (SCD) and beta thalassemia.

Sickle Cell Disease and Beta Thalassemia

SCD is one of the most researched monogenic (single gene-regulated) disorders in terms of gene therapy. Commonly known as sickle cell anemia, it is a genetic blood disorder in which a lack of healthy red blood cells inhibits oxygen transport.[32] Beta-thalassemia is another condition that is very similar to sickle cell anemia; it is caused by beta-globin gene deficiency (which controls hemoglobin production). SCD is caused by defective hemoglobin, specifically aberrant hemoglobin S. SCD treatment utilizes an ex vivo strategy in which stem cells are extracted and edited outside the body. Bone marrow stem cells are taken out and edited using CRISPR to render *BCL11A*, an HbF synthesis suppressor, inactive. This is done by giving patients autologous CD34+ cells. After this, chemotherapy eliminates disease-causing stem cells, and billions of edited stem cells are put back into the patient's bloodstream intravenously (Frangoul, 2021 #68).

Two patients in preclinical trials showed promising treatment results. Initial results from a follow-up of the first two patients treated with CTX001 showed the intended CRISPR-Cas9 editing of *BCL11A* in long-term hematopoietic stem cells, with durable engraftment, high levels of HbF expression, and elimination of vaso-occlusive episodes or need for transfusion. The generalizability of these early results to other patients with TDT and SCD should be determined. The preliminary results support the experimental testing of CRISPR-Cas9 gene editing approaches to treat genetic diseases. As a result, more ongoing trials, including CLIMB THAL-111 and CLIMB SCD-121, are looking to treat patients with both SCD and beta thalassemia using CTX001. CRISPR Therapeutics and Vertex Pharmaceuticals are running this trial in Europe and Canada. The CLIMB-111 Trial in beta thalassemia involves the treatment of 15 patients. All patients showed rapid and sustained increases in total hemoglobin after a three-month follow-up with CTX001 dosing. The results demonstrated that patients treated for SCD or beta thalassemia show normal to near-normal hemoglobin levels, where at least 30% (SCD) or 40% (beta thalassemia) of hemoglobin is HbF. Molecular tests on bone marrow from each patient a year or more after the treatment showed the continued presence of genome-edited cells. One patient with beta thalassemia experienced severe immune reactions to the treatment, which was resolved. No other serious adverse events were observed, and side effects seemed to be related to chemotherapy and not to genome-editing. The study is estimated to be completed in October 2024. Other clinical trials are looking to experiment with base editing, which is a form of genome editing involving a direct change in single DNA letters, to turn on HbF.[33,34]

LCA10

Another monogenic genetic retinal disease that causes blindness in children is Leber congenital amaurosis (LCA). The FDA authorized the gene therapy Luxturna in 2017, making it the first licensed gene therapy in the United States. Luxturna works exclusively in patients with RPE65 genetic mutation and viable retinal

cells. The treatment, which costs $850,000 for a single treatment, works by delivering a normal copy of the RPE65 gene via a viral vector. Alternatively, because this technology is more effective and easier to engineer, CRISPR/Cas-9-based technologies could be less expensive and more effective solutions to the disease.[35]

Over the years, low-vision aids were utilized to correct visual abnormalities, but it does not offer a long-term solution. Ruan and Barry24 highlighted that an AAV-mediated subretinal injection was utilized in gene editing by inserting a normal copy of the CEP290 gene. The CRISPR-Cas9 technology was employed with the drug EDIT-101, utilizing an AAV5 vector that generates a full-length, functioning CEP290 protein as opposed to a short, broken version of the protein. The procedure involved normal CEP290 protein splicing. The CRISPR-Cas9 technique improved the management of GRK1 promoters, which mediate transgene expression in rod and cone photoreceptors and is essential for treating LCA. This technology contributed to the improvement of retinal viral gene therapy; improved outcomes are anticipated in the coming years. The lack of a defined and dependable delivery route for the treatment is a limitation of the current technology. An adverse immune response could be triggered by a disruption in genetic material delivery.[36]

This condition was studied extensively. Since 2007, clinical trials targeted epithelium-specific 65kDa to study retinal pigments.[37] In 2019, Editas Medicine commenced CRISPR clinical trials, while Editas Medicine conducted BRILLIANCE, a two-phase clinical trial of EDIT-101. The 34-participant study will end in March 2024. Phase 1 is nearly complete, with a positive safety profile observed for 15 months and mild adverse events associated with retinal injection. Phase 1 trials showed gene editing and visual improvements. EDIT-101 could treat LCA10 diseases and create a paradigm for future in vivo gene editing systems.[38]

Cancers

CRISPR-Cas9 is being explored to eliminate tumors. This approach aims to modify white blood cells to be more resistant to cancer by optimizing the immune system. T cells are white blood cells that play an important part in the immune system by recognizing other cells as safe or threatening. These T cells are genetically altered to better attack cancers in Chimeric antigen receptor T-cell immunotherapy (CAR-T). Given how tightly regulated the immune system is, some T cell receptors act as "checkpoints" to decide whether an immune response occurs. Cancer cells often camouflage themselves as safety signals to mislead these "checkpoint" T cells into ignoring them. One key receptor is PD-1, which interacts with PD-L1 on another cell to signal that it is "safe." CRISPR is employed in CAR-T immunotherapy in a process known as checkpoint inhibition, which ensures that the "off" signal is not transmitted, allowing T cells to attack cancer cells effectively. An ex vivo approach is used in the treatment process, in which the patient's blood is extracted and engineered in a lab. This strategy makes it easier to administer genome editing tools to target cells; however, the treatment is longer and more expensive. The primary disadvantages of CAR-T immunotherapy originate from a side effect known as cytokine release syndrome (CRS), in which toxic cytokine quantities are released. Cytokines are small proteins that enable the immune system conduct its role, but when in excess, they can induce a widespread inflammation. According to published data, CRS of any grade occurred in 42–93% of patients with B-cell non-Hodgkin lymphoma undergoing CAR T-cell therapy, with grade 3 toxicity occurring in 2–22% of patients.[39]

There are currently eight clinical trials exploring CAR-T immunotherapy with CRISPR Cas-9. Notably, the CARBON clinical trial by CRISPR Therapeutics showed positive results from its Phase 1 trial, in which CTX110 was used to treat relapsed or refractory CD19+ B-cell malignancies. There was a 58% overall response rate and 38% complete response rate in large B-cell lymphoma with a single dose of CTX110. These response rates and durability were similar to already approved autologous CD19 (an antigen similar to PD-1) CAR-T therapies, as the onset of CRS was low.[40,41] Another clinical trial by CRISPR Therapeutics is studying CTX130, a CRISPR Cas-9 ex vivo medicine for patients with refractory T- or B-cell malignancies (COBALT-LYM) and patients with renal cell carcinoma (COBALT-RCC).[40] The Phase 1 trial for COBALT-

LYM has released ongoing data, showing a 70% overall response rate, 30% complete response rate, and a clinical benefit of 90% for patients.[42]

Leukemia

Leukemia is a blood cancer that affects both the lymphatic system and bone marrow. Proliferation in the lymph nodes and spleen causes the body to produce an excessive amount of white blood cells.[37] Chronic myeloid leukemia (CML) and other variants are being studied using gene editing to eliminate cancer-causing oncogenes, particularly in single oncogenic cancers like CML. Current treatments involve the use of rationally designed inhibitors of mutated proteins. However, an oncogene that remains unaltered would force patients to be on medication for an extended period of time.[43]

CRISPR Cas-9 is being investigated to offer a safe platform for treating CML by targeting the tyrosine-kinase BCR/ABL1 oncogene, which is responsible for CML development. Over the last few years, the treatment was evaluated and refined substantially. García-Tuñón, Hernández-Sánchez, Ordoñez, Alonso-Pérez, Álamo-Quijada, Benito, Guerrero, Hernández-Rivas, and Sánchez-Martín[44] demonstrated that the CRISPR-Cas9 system effectively abrogated the BCR/ABL1 oncogene, which is required for further CML exploration. This was accomplished using a CML xenograft mouse model in which an edited CRISPR suppressed tumorigenic activities, implying that no tumors originated from edited cells.[44] Later, Chen and Hsieh33 used a CRISPR/Cas9 vector to disrupt the ABL1 gene in human cells. However, it was observed that ABL1 disruption did not trigger important consequences; thus, a new approach, which was based on the use of another guide, introduced a large gene deletion. Martinez-Lage and Torres-Ruiz34 proved (through a mouse model) that a frameshift mutation in the BCR/ABL1 oncogene resulted in an 88% decrease in tumor size, so the CRSIPR-Cas9 method was implemented for editing the oncogene. Furthermore, another study by Vuelta and Ordoñez35 provided a definitive proof of CRISPR/Cas9-edited leukemic stem cells in patients with CML, thereby demonstrating how CRISPR/Cas9 technology can easily be used to destroy driver oncogenes.

CRISPR/Cas-9 clinical trials for CML are expected to commence in the near future. The only treatment for leukemia is only for acute myeloid leukemia (AML), which is Intellia Therapeutics' NTLA-5001. NTLA-5001 is an ex vivo T-cell therapy under investigation for all forms of AML. The study's phase 1 trial has 54 participants and is expected to be completed in September 2025. Other treatments for leukemia, including CAR-T immunotherapy, which is used to treat B-cell leukemia and lymphoma, are being investigated through clinical studies.[45]

Lymphoma

Lymphoma is a cancer of the lymphatic system that targets the bone marrow and thymus gland and is characterized by lymph node enlargement and weight loss.[46] Patients with lymphoma have a survival span of less than five years from diagnosis.[47] Some researchers indicated that CRISPR/Cas9 helps enhance lymphoma immune evasion by targeting deregulated genes, including the B-cell marker CD19 in the tumor microenvironment.[48] It was revealed that 15 of 17 patients with T-cell lymphoma could be examined as of December 6, 2021. The overall response rate was 71%, with 29% of patients fully recovering. The success rate was higher than that of other immunotherapies, and the procedure could be used in the future. It achieves results by reducing the sensitivity of B-cell lymphoma cells and preventing drug dependence among patients. This would assist in eliminating lymphoma cells and preventing their spread to other human organs. CRISPR/Cas9 revolutionized lymphoma clinical trials by reducing the development of tumors after T-cell engineering.[49] These cell-based therapies help repair genetic alterations, which will lead to immune response stimulation. Quazi38 outlined that the technique’s efficacy moved researchers away from ZFNs, TALEN, and other gene editing methods. The CRISPR model will be essential in determining how proteins contribute to aggressive lymphoma and how they express themselves. It is expected to influence future research directions as researchers continue to specialize in gene editing techniques. The significant advantage of clinical trials is that they aid in determining the

efficacy of cell-based therapies. These findings indicate that there is a considerable potential for managing tumor malignancies like lymphoma in the future. However, it is difficult to prove their effectiveness in graft-vs-host disease. These trials are still ongoing in their early stages, and the treatment may be ineffective.

Lung Cancer

Lung cancer causes uncontrolled cell proliferation in the lungs. When there is an overgrowth, it impacts how the lungs take in oxygen and exhale carbon dioxide. The disorder can affect individuals who never smoked, but smoking places an individual at a higher risk. There were 148,869 deaths associated with lung cancer in 2016, making it the most lethal form of cancer.[48] An early detection of the condition is crucial since it reduces the risk of mortality; thus, lung cancer screening is widely recommended.

In 2016, a patient with lung cancer became the first person to receive a CRISPR-based therapy as part of a Chinese clinical trial at Sichuan University. The trial involved injecting PD-1 edited T cells into 12 patients with non-small-cell lung cancer. This approach did not use CAR-T therapy because it is not currently a treatment option for lung cancer. The main goal of this and a similar clinical trial in the US was to assess the safety and side effects of the treatment, rather than its effectiveness.

In April 2020, the researchers reported that the treatment was generally safe to administer, with minor side effects such as fever, rash, and fatigue. The intended genetic edit was present at a low efficiency, with a median of 6% of T cells per patient being edited before infusion. There were also low frequencies of off-target effects, which are unintended changes at other locations in the genome, mostly in non-coding regions. On-target effects, or unintended changes at the intended target site, were more common, with a median of 1.69%. Eleven out of the 12 patient volunteers had edited T cells present at low levels two months after the infusion. Patients with higher levels of edited cells had less disease progression.[50,51]

A recent study conducted by Christiana Care's Gene Editing Institute in the US demonstrated how to recognize and assess the broad biological impact of gene editing on targeted tissues, where edits were intended to entirely disable a specific segment of the genetic code. This study demonstrated the safety and efficacy of CRISPR gene editing within inpatient treatments. Other studies proposed using CRISPR to deactivate or modify a master regulator gene to inhibit it from expressing a protein that inhibits chemotherapy effects and increases the likelihood of a successful lung cancer chemotherapy.[52]

Future Technologies

CRISPR Cas9 is currently the most widely used genetic engineering tool in clinical trials. However, a growing body of evidence points to a safer technological path via new CRISPR technologies that are being discovered at a rapid pace. For instance, the SuperFi-Cas9 variant discovered by UT Austin has the potential to reduce off-target mutations by 4000 times while still working as fast as naturally occurring Cas9. Mutations to stabilize structures, which were discovered in March 2022 using cryo-electron microscopy, were used to solve mismatch tolerance, the issue in which 18/20 nucleotides were identified as a match. Therefore, the guide RNA made the connection, despite being in a different location in the genome. These mutations effectively inhibit a connection during a DNA mismatch, ensuring that the guide RNA only pairs with 19 or 20 matching nucleotides for Cas9 to cut. The size of the CRISPR/Cas complex is another limitation of the CRISPR technology.[53] Stanford seeks to solve this problem by pioneering miniCRISPR, which is said to be a Swiss Army knife in the molecular scissors standard of Cas9 technology. Stanley Qi of Stanford designed an efficient miniCRISPR system, wherein CRISPR-associated proteins (typically Cas9 or Cas12a) consist of 1000 to 1500 amino acids, and Cas12f (or Cas14) only contains 400 to 700 amino acids. Because of the reduced size of such delivery mechanisms, the major hurdle in fitting the protein complex within smaller human cells was solved. The technology was already tested for genes related to

HIV infection {Das, 2019 #69}, anti-tumor immune response {Alishah, 2021 #70}{Rupp, 2017 #71}, and anemia {Richardson, 2018 #72}{Román-Rodríguez, 2019 #73}.

Conclusion

CRISPR allows an accurate and diversified genome editing. These adaptive technologies, called "universal tools," revolutionized biological sciences and made important research discoveries possible. CRISPR will likely be utilized in healthcare institutions to cure human diseases, including cancers. These technologies may be employed for therapeutic purposes if novel techniques of introducing genome engineering tools into cells and enhancing their editing capacity are developed. In vivo and ex vivo tumor research uses CRISPR/Cas. Several ongoing clinical studies aims to improve the efficacy of cancer treatments. These technologies could deliver precise and desired genome editing in the modern era of medicine.

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Authors

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COVER LETTER

16th January 2023
Dr. Richard Beal
Editor
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Dear Editor:

Please find enclosed our manuscript entitled "CRISPR in Medicine: A Review of the Technology, Challenges, and Applications in Clinical Trials," which we request you to consider for publication as a *Review Article* in the *International Journal of High School Research*.

With the gene as the basic unit of heredity, the ability to make specific modifications in a genome is essential to combat diseases. One of the most promising techniques for accomplishing this goal is gene therapy, which has the potential to cure cancers, infections, and genetic disorders. There are currently relatively few FDA-approved treatments that use this technology, and they are all primarily ex vivo, making it exceedingly exclusive and expensive. Cas9, which is the most extensively used gene-editing nuclease, has a significant promise for the treatment of cancer, viral infections, and genetic disorders, among other conditions. CRISPR allows an accurate and diversified genome editing. Recent research also identified additional CRISPR-Cas system varieties, including superFi CRISPR or miniCas, which can be applied for gene editing. Despite the quick advancements in basic research and clinical trials, numerous fundamental issues, such as editing effectiveness, relative delivery difficulties, off-target consequences, and immunogenicity, present major ongoing obstacles. Therefore, this review highlights the recent applications and challenges in the CRISPR-Cas9 technology in clinical trials.

This manuscript has not been published elsewhere and is not under consideration by another journal. We have approved the manuscript and agree with submission to the *International Journal of High School Research*. There are no conflicts of interest to declare.

We believe that the findings of this study are relevant to the scope of your journal and will be of interest to its readership. The manuscript has been carefully reviewed by an experienced editor whose first language is English and who specializes in editing papers written by scientists whose native language is not English.

We look forward to hearing from you at your earliest convenience.

Sincerely,

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