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# CRISPR-Cas9 GENOME-EDITING TECHNOLOGY: A TRANSFORMATIVE TOOL FOR CURING HUMAN DISORDERS

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## ABSTRACT

CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats", which form the foundation of an ancient immune system in single-cell organisms, especially bacteria and archaea. It has shown a positive therapeutic potential by modifying the genetic mutations responsible for incurable genetic disorders. CRISPR has several benefits over other gene-editing tools, as it is convenient, inexpensive and efficient. Despite therapeutic potential, CRISPR can also help to make animal models to study disease development and progression, and also to study the effect of new drugs. CRISPR can help to treat several genetic diseases, including cancers, allergic diseases, immunological disorders, cardiovascular diseases, viral infections, cystic fibrosis, muscular dystrophy, Huntington's disease, metabolic disorders, blood disorders and eye-related disorders. CRISPR can also help to treat

celiac disease by modifying the gliadin content in the wheat crop. Pharmaceutical and therapeutic companies are developing CRISPR therapies against genetic diseases and are concerned about the off-target effects of CRISPR, along with its ethical, legal and safety concerns. CRISPR laws are more stringent in the European Union than in the United States, which critics believe could hamper the investment in this research. The future holds great promise for CRISPR in treating diseases, developing medicines and fostering agricultural production.

## INTRODUCTION

"Clustered Regularly Interspaced Short Palindromic Repeats" (CRISPR) is a succession of recurring DNA patterns in the genome of bacteria and archaea, which forms a primitive defence system that helps the bacteria to recall the DNA sequence of the invading viruses by integrating the viral DNA series within the CRISPR system (Ganger et al., 2023). Cas9 protein is a powerful gene editing tool that functions like molecular scissors, cutting any desired DNA molecules that match the sequence of the nucleic acids stored in the CRISPR patterns (Irfan et al., 2024). With progression in the field of genetic engineering, CRISPR can now cut any desired DNA pattern simply by providing Cas9 the required template. CRISPR has benefits over other gene editing tools that require making a protein for each DNA modification, but in CRISPR, only a single Cas9 endonuclease can do the task, merely by facilitating it with a single guide RNA (sgRNA) sequence that is far easier to synthesise. So, it is convenient, cost-effective and less time-consuming (Asmamaw et al., 2021). CRISPR has also laid the foundation for the introduction of more precise gene editing techniques, base editing and prime editing. Base editing is used to replace one DNA base with another, like cytosine to thymine, without breaking the DNA double helical structure. It blocks accidental mutation and also can help to remove point mutation, as in genetic diseases (Sichani et al, 2023). Prime editing is known to be a search-and-replace type of editing. It consists of insertion, removal or replacement of nucleotide sequences without disrupting the DNA duplex, utilising a pair of catalytically impaired Cas9 and reverse transcriptase (RT) that is performed by an engineered prime editing guide RNA (pegRNA). It can be applicable in the correction of several high-precision genetic variations (Chen et al., 2023).

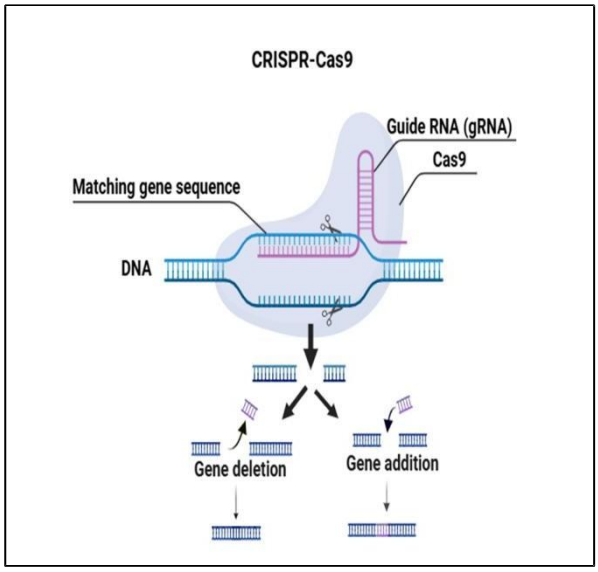


**Figure 1.** CRISPR-Cas9 genome editing technology mechanism

CRISPR makes use of a guide RNA (gRNA) to localise the Cas9 nuclease to DNA sequences of interest. It cuts certain genes of the DNA at exact positions in the exact

sequence in order to delete them and insert them in their place, followed by the cells' natural repair process.

Moreover, the uses of CRISPR do not just lie in the treatment of human genetic diseases; this technology may assist researchers in making faster and more precise diagnoses of mutations and diseases (You et al., 2022). With CRISPR, researchers can create animal models that have human-unique features or immunity to make them more relevant for the study of how disease progresses in a human being. Specifically, the mouse models of acute myeloid leukaemia (AML) are engineered by provoking genetic alterations in the hematopoietic stem cells (HSC) through CRISPR-Cas9 (Kurtz et al., 2022). It can also help tackle global warming by developing methane-free cows (Subedi et al., 2022) and crops which are more disease resistant, have higher yield production and grow faster (Ahmad et al., 2021). The CRISPR technology can be employed to create gluten-free wheat that such patients with coeliac disease could eat (Sanchez et al., 2024). It is not only that the CRISPR may assist in the conservation of the endangered species, but it can also help in resurrecting extinct species. One de-extinction company, Colossal Biosciences, is utilising the application of the CRISPR-Cas9 gene-editing tool on their current three projects that involve the de-extinction of the mammoth, the thylacine, also known as the Tasmanian tiger, and the dodo (Reymers et al., 2025).

The CRISPR therapies are still facing limitations, including off-target effects and other unpredictable efficacies of using genome editing. Safety strategies and long-term consequences of these CRISPR technologies are still under review. Future studies are needed to ensure the safe practices of such genetic procedures in human beings (Bhardwaj et al., 2024). The CRISPR technique also has a lot of ethical and legal issues, besides which it is already under tight restrictions in the European Union. Genetically modified plants and crops with the CRISPR technology in Europe fall under the stringent genetically modified organisms (GMO) regulations that involve a lengthy and hectic license procedure (San-Epifanio et al., 2023). Nevertheless, the American laws on CRISPR are more flexible (Heinrich et al., 2024). Critics believe that these restrictions can affect investment and funding in the genetic engineering field, and Europe can lag behind in this advancement and research.

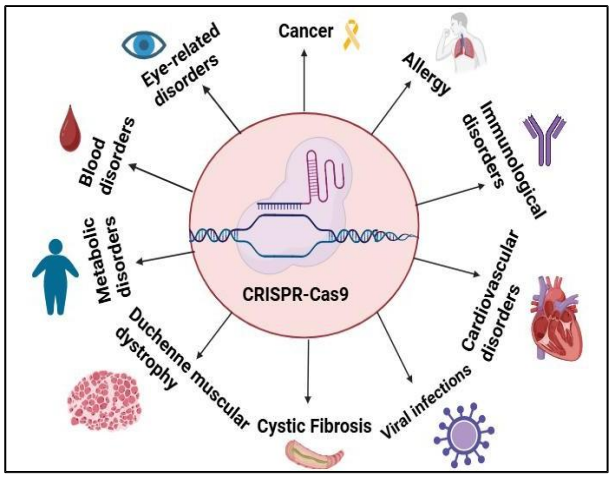


**Figure 2.** Applications of CRISPR-Cas9 in the treatment of human genetic disorders

CRISPR-Cas9 gene-editing tool has proved its therapeutic potential against genetic diseases by correcting the mutated genes responsible for genetic disorders via gene deletion, insertion or substitution.

**CRISPR in Treating Diseases**

**Cancer**

Cancer is a major cause of mortality worldwide, accounting for nearly 10 million fatalities in 2020 (Workie et al., 2023). It is the unchecked development of abnormal cells in the body's tissues or organs. Cancer is a complex disease with no universal cure. There are more than 200 types of cancers and each with its unique mutations and adaptability that make it difficult to formulate a single cure plan (Cancer Research UK, 2023). Traditional chemotherapy is not target-specific, and more than 90% of deaths recorded from cancers are

due to chemotherapeutic drug resistance (Bukowski et al., 2020). CRISPR will have the ability to alter the future of cancer research and treatment. It may assist in screening, diagnosis and treatment, as well as provide treatment for cancer by stimulating the identification of the genes or proteins involved in the mutation. Cyclin-dependent kinases (CDKs) play a central part in cell cycle control and other cell functions. Thus, tumorigenesis can occur due to a disruption in the activation of CDKs in any way. This denotes that CDKs are a productive treatment locus to heal the cancer as reported by Zhra et al. (2025) and Tiwari et al. (2023) that the CRISPR-Cas9 can be useful in formulating the therapy of osteosarcoma and triple-negative breast cancer (TNBC) by hushing the CDK11 and CDK7 genes, respectively.

SHC binding protein-1 (SHCBP1) contains a protein SH-2 (Src homology 2) domain represents a key controller of cell proliferation. Overexpression of SHCBP1 gene is known in numerous illnesses, including cancer. Hence, it is a useful anti-cancer therapeutic target and biomarker. It is found that SHCBP1 gene can be knocked-off using CRISPR in breast cancer cells, leading to growth inhibition and apoptosis of the cells (Sharma et al., 2021). The members of the Kelch-like gene family (KLHLs) are involved in different diseases, encoding several classes of proteins. The mutation that occurs in the KLHL genes is associated with cancer development and advancement. Tumour growth and migration, and even apoptosis of cancerous cells were inhibited as a result of the knockout of Kelch domain containing 4 gene (KLHDC4) with the assistance of CRISPR in nasopharyngeal carcinoma cell line (Kanbar et al., 2024).

The major impediment to the chemotherapy of cancer is due to chemotherapeutic drug resistance. The multidrug resistance 1 (MDR1) gene encodes P-glycoprotein (P-gp), a transmembrane protein which acts as a multidrug efflux pump, and it plays a role in the removal of chemotherapeutic drug molecules out of the cells, hence producing resistance. The same is seen more recently in how targeted MDR1 regulation with CRISPR can re-sensitise cells to chemotherapy; indeed, Vaghari-Tabari et al. (2022) have demonstrated the same in clinical practice with how the sensitivity of osteosarcoma cells was restored using CRISPR.

One of the four primary cancer treatment modalities, together with surgery, radiotherapy, and chemotherapy, is immunotherapy (Rudra et al., 2023). The concept of CRISPR, when applied to immunotherapy, is mainly focused on chimeric antigen receptor (CAR) T and engineered T-cell receptor (TCR) cell therapies (Wellhausen et al., 2022). China carried out the first clinical trials of CRISPR using T cells extracted from a patient with Stage 4 lung cancer (Song et al., 2024). CRISPR was applied to the removal of the genes that code the programmed cell death protein 1 (PD-1) receptor in immune cells. PD-1 enables the tumour cells to bind and prevent the immune response against cancer. PD-1 ligand is located on the surface of malignant cells. When PD-1 binds to its ligand, PD-L1, this inhibits the actions of cytotoxic T lymphocytes (Han et al., 2020). Immune checkpoint inhibitors restore an anti-tumour immune response by interfering with the interaction between PD-1 and PD-L1. Genetic modification of autologous T lymphocytes introduces chimeric antigen receptors (CARs) into T-cells, causing the T-cells' surface to express CARs. After re-infusion, these CAR-modified T cells will multiply and attach to the tumour-specific antigens, thereby commencing the specific destruction of malignant cells. (Li et al., 2020). Cytokine release syndrome (CRS) is a known adverse effect of CAR T-cell therapy and may trigger a propagated organ impairment (Xiao et al., 2021). Compared to TCR therapy, which is targeted, CRS, in turn, is less

frequent (Shafer et al., 2022). As a result, there is an upsurge in the preference for TCR therapy in the clinic. However, improvements in genomic engineering, especially as CRISPR/Cas9, have boosted the efficacy and safety of natural killer (NK) and CAR T-cell therapies, by reducing off-target effects and increasing resistance to the immunosuppressive effect produced by tumours (Amiri et al., 2024). In the United States, CRISPR Therapeutics has developed CRISPR gene-edited T cell therapies that target CD19 and CD70 proteins, which are the biomarkers for B lymphocyte development and are in clinical trials (Feng et al., 2024).

**Allergy and Immunological Disorders**

Allergic diseases encompass a variety of conditions resulting from the immune system's hypersensitivity (Shamji et al., 2021). CRISPR can also be used to treat allergic and immunological disorders. Melanoma cell adhesion molecule (MCAM), another name for the MUC18 gene, encodes for a cell surface glycoprotein. Over-expression of these genes in the alveolar macrophages is noted in asthma and chronic obstructive pulmonary disease (COPD) caused by viral or bacterial infection, which could aggravate inflammation and airway damage (Avci et al., 2022). CRISPR can help in the removal of MUC18 gene that could cause a significant reduction in the levels of pro-inflammatory chemokine, interleukin-8 (IL-8), produced by the nasal airway epithelial cells (AECs) (Orr, 2023). In the therapy of cancer, checkpoint inhibitors are innovative targets. CRISPR can help in modifying the PD-1 receptor protein in autologous T-cells that could cause upregulation of interferon (IFN)-Y, hence resulting in improved cytotoxicity (Xu et al., 2022).

One primary immune deficiency condition is X-linked hyper immunoglobulin M (IgM) syndrome in which B cells face difficulty in transitioning from making IgM antibodies to other Ig classes; IgA, IgE and IgG. This disease is due to the abnormal genetic modifications in the CD40LG gene (Kemme et al., 2021). CRISPR-Cas9 can help to correct these genetic mutations in the CD40 ligand and can restore T-cells' interaction with B-cells, leading B-cells to switch classes (Rogers & Cannon, 2021).

CRISPR-associated gene-editing tool has a prospective to use in the prevention of allergic diseases because they can rectify mutations of Janus kinase 3 (JAK3) gene, which codes JAK3 proteins, one of the critical protein tyrosine kinases in cytokine signalling and in the immune process that mediates the differentiation of T-cells and NK cells (Önal, 2024). Mutations in JAK3 may cause a severe combined immunodeficiency disease (SCID), where the immune system is deeply damaged. The incomplete functioning of JAK3 protein will also prevent the transportation of chemical signals to the nucleus, disrupting the maturation of the lymphocytes and then the immune functions, leading to a decline in the number of functional immune cells: T-cells, B-cells, and NK-cells (Basheer et al., 2022). The technology that uses CRISPR promises to reclaim these unfavourable mutations and to enhance the ability of the body to guard against allergic disease. At the same time, studies are on course with the view of silencing or modifying the JAK3 gene once the condition of asthma has been initiated with a view of diminishing inflammatory reactions that are a product of immune activation (Georas et al., 2021).

**Cardiovascular Diseases**

Cardiovascular diseases (CVDs) represent a complex of disorders, the causes and development of which lie in the heart as well as the vasculature. A high level of serum cholesterol concentration is one of the major contributors to CVD. Encoded as proprotein convertase subtilisin/kexin type 9 (PCSK9), sequestering this protein, which alters the processing of low-density lipoprotein (LDL) receptors, thereby increasing their degradation.

This protein suppresses the elimination of low-density-lipoprotein-cholesterol (LDL-C) in the circulation (Bao et al., 2024). The increase in activity of the PCSK9 protein after the development of a gain-of-function mutation in the PCSK9 gene can cause significant increases in the level of LDL-C, thus leading to the significant increase in developing coronary artery disease (CAD), hypercholesterolemia, and atherosclerosis (Alavi et al., 2024). The current research proves that the CRISPR/Cas9 genome-editing machinery can be successfully used not only to cure CVDs but also genetic lipid disorders, as the research conducted by Angom et al. (2024) shows using the zebrafish model.

**Viral Infections**

Acquired immunodeficiency syndrome (AIDS) is a chronic abnormality which hinders the immune system, increasing the risks of infectious diseases and tumours. CRISPR can be used to delete the viral DNA integrated into the DNA of the helper T-lymphocytes infected by the human immunodeficiency virus (HIV). Though the current antiretroviral therapy can slow the progression of viral infection, it cannot perform eradication due to the quiescent reservoir of HIV (Hashmat et al., 2020).

The CRISPR-Cas9 technology has the potential to directly endow human beings with resistance against HIV by replicating the natural resistance system that exists in the human body against HIV. The CCR5 gene, which codes for chemokine receptor type 5 protein that appears on the membranes of the leukocytes, is the major pathway that HIV uses to gain access inside the cell. In particular populations, there is a naturally occurring mutation (CCR5-delta32) which alters the structure of CCR5, thus eliminating it as a site of HIV binding. The latter is such a widespread variance that provides a background to a group of people whose resistance to infection seems to be innate (Tehranian et al., 2022). Antiviral gene product augmentation can be achieved using molecular editing, including CRISPR. In particular, genome editing may increase the expression of the apolipoprotein B mRNA-editing enzyme (APOBEC3G) and the tripartite motif-containing protein 5 alpha (TRIM5α) gene. APOBEC3G mediates mutations in the viral DNA, and TRIM5 alpha targets the viral capsid, thus preventing HIV-1 replication. All of them form the powerful host-derived restriction of HIV-1 infection (Salman et al., 2023; Somers, 2023). A clinical trial was the first in which dosing of a CRISPR-based gene-editing therapy, developed by Excision Biotherapeutics, was administered to a human in 2022. This agent, EBT-101, is the CRISPR gene-editing technology that is able to target and remove HIV viral DNA out of host immune cells. Incomplete results of the trial suggest that the treatment is safe and well-tolerated (Thomas et al., 2023), but those patients who stopped using antiretroviral therapy (ART) early developed viral rebound (Letchumanan et al., 2025).

Since its discovery, CRISPR has raised a lot of controversy; one of the most disputable events took place in 2018, when Dr. He Jiankui applied the method to carry out modifications on the CCR5 gene in embryos of humans, in an attempt to make them resistant to HIV, a move that eventually led to the creation of what has been termed as the CRISPR babies. The episode raised a major public and academic debate over the ethical and moral use of gene-editing devices because the inherited changes may potentially persist into future generations (Rose et al., 2019).

Hepatitis is an inflammation of the liver and is largely due to agents that are viral in nature. Hepatitis B virus (HBV) and the hepatitis C virus (HCV) represent some of the main etiological agents in the world, causing the most significant liver pathology (Ou et al., 2024). CRISPR-Cas9 has also been noted as one of the strategies of anti-HBV therapy in a

way that it can cleave the viral DNA to reduce HBV replication and disease progression (Yao et al., 2024). Similarly, CRISPR technology can be used to inhibit the replication of HCV by using the protein *Francisella novicida* Cas9 (FnCas9) that specifically binds and targets the degradation of HCV RNA (Bartosh et al., 2023).

Human papillomavirus (HPV) is a type of pathogen that is a double-stranded DNA virus commonly known to precipitate sexually transmitted infections (STIs), and it is the chief pathogen in the creation of cervical cancer (Choi et al., 2023). The process of tumorigenesis in the cervix involves the inactivation of the E6 and E7 genes of HPV, which impairs the physiological role of the p53 and retinoblastoma (Rb) tumour suppressor proteins, indicating the facilitation of unregulated cell growth (Bønløkke et al., 2024). CRISPR-Cas9 is a future form of therapy against HPV infections. Such an approach uses Cas9 nuclease in combination with a guide RNA (gRNA), which chooses and cuts HPV DNA at specific sites and disarms the viral oncogenes (Kermanshahi et al., 2025).

Epstein-Barr virus (EBV) or herpesvirus 4 is a representative of the Herpesviridae family and is described by the presence of a double-stranded DNA genome. It is involved in infectious mononucleosis, also known more often as glandular fever (Naughton et al., 2021). CRISPR/Cas9 gene-editing technology has been used to insert a break in the intended sequences of the BART promoter gene, which contains viral microRNAs (miRNAs) (Najafi et al., 2022). In addition, cellular receptor Ephrin receptor tyrosine kinase A2 (EphA2) is a transmembrane glycoprotein serving as a potential entry location of EBV into host cells, that is, mainly epithelial cells, which makes EphA2 a viable target in CRISPR-based therapy (Zahedipour et al., 2024).

**Celiac Disease**

Celiac disease (CD) is an inherited autoimmune disorder which impairs the mucosa of the small intestine as a result of hypersensitivity to gluten. Patients with CD cannot consume gluten foods like wheat, barley and rye. They have to stick to a lifelong gluten-free diet to avoid complications. This diet is not very cost-effective, as the gluten-free products available in the markets are very costly (Afzal et al., 2024).

Unlike other genetic diseases where CRISPR is used to alter genes within the patient's own cells, CRISPR, in the case of CD, targets gluten-containing crops instead of the patients. Several studies have been made so far that have demonstrated the use of CRISPR to decrease the gliadin proportion in wheat by modifying its genome. Notably, one study reported a reduction of 97.7% in the gluten content of wheat through targeted gene editing (Sánchez et al., 2024). Researchers are trying to make gluten safe for celiac patients by removing the specific parts of antigens in the gluten protein called epitopes which trigger the immune response (Jouanin et al., 2020). This task is not as simple as it sounds because wheat has a very complex genome. Wheat is a hexaploid plant with six sets of chromosomes, that is, having three sets of homologous chromosomes from ancestral genomes A, B and D. This means that to modify only a single gene, you have to alter all three homologous copies, one from each genome, and to make it even more complex, some chromosomes may have multiple copies of the same gene (Levy & Feldman, 2022; Cui et al., 2023).

**Cystic Fibrosis**

Cystic fibrosis (CF) is an autosomal recessive single-gene disorder that is typified by gradual destruction of the lungs, pancreatic gland, and gastrointestinal tract, among other systems. The disorder interferes with the normal physiological mechanism of mucus

clearance of the pulmonary airways, thus leading to the occurrence of chronic airway obstruction episodes, frequent respiratory infections, and other respiratory complications (Chen et al., 2021). The CF genetic mutation can even be passed on to future generations.

CF is a hereditary condition that arises because of a defect in CFTR gene. The gene encodes the cystic fibrosis transmembrane conductance regulator (CFTR), which is a protein that generally controls the regulated passage of chloride and water across the membrane through the activity of the protein as a channel. Once the CFTR gene has been mutated, the protein no longer has the ability to carry out such functions, leading to the clinical manifestation of cystic fibrosis: chronic cough with mucus, nasal polyps, wheezing, shortness of breath, frequent lung infections, as well as chronic gastrointestinal disorders of constipation and large, greasy, and foul-smelling faeces (Lopez-Valdez et al., 2021). As of now (to the best of our knowledge), there were over 700 different mutations in the CFTR gene, so the idea of developing a targeted pharmacotherapy for each mutation is nothing short of impossible (Caraco et al., 2025). The mutations can be corrected (with a patient-specific manner) with genome-editing technologies, notably CRISPR. In order to better understand the pathophysiology of CF, the mutations of the CFTR gene have also been added to sheep models through CRISPR-Cas9 (Viotti Perisse et al., 2021).

In 2020, the Dutch researchers used adenine base-editing, an editing tactic of precision genome-editing that combines CRISPR to cure CFTR mutations without inducing off-target effects in CF cell in vitro models (Geurts et al., 2020). CRISPR Therapeutics are also in a joint venture with Vertex Pharmaceuticals to pioneer the first CRISPR-based product to enter clinical development to treat CF (Altshuler & Davies, 2024).

**Muscular Dystrophy**

Duchenne muscular dystrophy (DMD) is an X-linked disorder, which is a group of extraordinarily uncommon neuromuscular diseases that cause the skeletal muscles to weaken and degenerate over time (Muntoni et al., 2024). There are various types of this disease that are determined by the affected skeletal muscle groups, the degree of weakness, the speed with which the disease progresses, and the age when the typical symptoms of muscular dystrophy emerge (Thomas et al., 2024). It results from a mutation in the DMD gene that encodes for dystrophin protein, helpful in normal muscle functioning by protecting muscle fibres from damage during the periods of relaxation and contraction (Lopez et al., 2021). Current therapies are restricted to a small number of patients, with primary treatments focusing mainly on dealing with the symptoms and slowing its advancement (Brown et al., 2023). DMD can be caused by multiple genetic mutations, these may be deletions, duplications or point mutations, accounting for nearly 60-70%, 5-15% and 20% of genetic mutations, respectively (Dongsheng et al., 2021). Over 3000 genetic mutations have been recognised so far (Mirza & Karim, 2024). However, mouse studies have evident that CRISPR/Cas-9 can prove to be beneficial and ground-breaking in treating DMD. In 2018, a team of researchers in the United States developed CRISPR Cas-9, utilising gRNAs to target mutation hotspots in the dystrophin gene of a mouse model (Long et al., 2018). Efficacy of CRISPR in correcting the DMD gene mutation can be improved by using self-complementary adeno-associated virus (scAAV) as a delivery vehicle (Zhang et al., 2022). Vertex Pharmaceuticals is focused on researching and developing gene therapies that could target the genetic modifications in the dystrophin gene, hence restoring the expression of the dystrophin protein (Vertex Pharmaceuticals, 2025). However, in

November 2022, a 27-year-old patient with DMD died because of the severe cardiopulmonary toxicity, acute respiratory distress syndrome and heart attack, in a CRISPR trial led by Cure Rare Disease, a non-profit biotechnology company in Boston, when targeting a rare DMD mutation (Lek et al., 2023).

**Huntington's Disease**

Huntington's disease (HD) is an inherited autosomal neurodegenerative condition that causes the gradual disintegration of nerve cells in the brain, impacting reasoning, memory and movement. It presents a triad of psychiatric, cognitive and motor symptoms. This disorder is caused by aberrant repeats of specific DNA sequences inside the huntingtin gene, also known as HTT gene, which is responsible for various cellular functions (Stoker et al., 2022).

The HD would have very specific safety issues associated with the application of CRISPR-based gene-editing tactics because any off-target effects in the brain can trigger catastrophic consequences. To alleviate such risks, it has suggested modifications of the base CRISPR/Cas9 platform. Some versions of CRISPR/Cas9 have also been developed; in 2017 researchers at the Institute of Bioorganic Chemistry, Poland generated a variant of CRISPR/ Cas9 by coupling it with a nickase enzyme, which nicks a single strand of DNA at the specific genomic locus where editing occurs but does not completely cut the double helix, making the editing more specific (Dabrowska et al., 2018). Another safeguard introduced in 2018 was a CRISPR/Cas9 set-up with a self-destruction system that shuts down Cas-9 protein after a specified time of its activation or in response to a certain trigger (Wang et al., 2020). At the University of Illinois Urbana-Champaign in 2022, instead of Cas9, CRISPR/Cas13, which has the same mechanism of action but is specific to RNA, was used by targeting and cutting RNA. In this approach, the method was implemented in a mouse model to remove RNAs that carried the mutant proteins that led to HD. Cas13 uses RNA instead of DNA, hence preventing the lifelong off-course mutations as RNA is short-lived and breaks down after some hours (Montagud-Martínez et al., 2024). Later on, in 2023, it was proved that this approach delayed the progression of HD in the mice and also prevented the death of some nerve cells (Morelli et al., 2023).

**Metabolic Disorders**

Hereditary tyrosinemia (HT) is an autosomal recessive genetic disease induced by the deficiency of fumarylacetoacetate hydrolase (FAH) enzyme due to a mutation in FAH gene. In this condition, the body faces difficulty in breaking down the amino acid tyrosine due to the FAH enzyme deficiency, which is vital for tyrosine metabolism (Thomas et al., 2025). The most common is the HT type I (HTI) that can cause chronic liver conditions, like hepatic cirrhosis, hepatic failure and liver cancer, as well as kidney damage due to toxin buildup (Ilyaz et al., 2024). The CRISPR has demonstrated therapeutic efficacy in this disease. Urban et al. (2025) showed the reconstitution of the FAH gene of HTI mice with the aid of CRISPR/Cas9. In addition, the genome editing therapy with CRISPR inhibited the development of liver diseases in HTI mouse model according to a study by Adlat et al. (2025), which implies that CRISPR is a safe genetic treatment for this illness.

Another disorder is the Hunter syndrome, also known as mucopolysaccharidosis type II (MPS II), which is a very rare and recessively inherited metabolic disorder present on the X chromosome that results in the progressive accumulation of large sugar molecules, which are also known as glycosaminoglycans (GAG), in tissues. The accumulation is attributed to the shortage of iduronate-2-sulfatase (IDS), an enzyme that is located in the lysosome and breaks down particular sugar residues. The disorder that occurs, in

consequence, is a multisystemic one and it involves the heart, lungs and the brain (Muenzer et al., 2021). The IDS gene codes IDS enzyme; defect in the IDS gene leads to abnormal deposition of GAG within the body. Sangamo Therapeutics announced the first in vivo zinc finger nucleases (ZFNs) clinical trial of Hunter syndrome in 2017, using an adeno-associated virus (AAV) vector to deliver the gene-editing technology directly to the bloodstream - the first time a genome-editing technology had been delivered in vivo to insert a functional copy of an IDS gene into the hepatic cells at the albumin locus. No significant side effects were noted in this treatment, suggesting that CRISPR is safe for treating genetic metabolic disorders (Li et al., 2020).

**Blood Disorders**

Blood disorders encompass a wide range of disorders, generally affecting the blood's ability to carry oxygen, clot or produce healthy red blood cells. These include thalassemia, sickle cell disease and haemophilia, etc (Thakare et al., 2021).

Sickle cell disease (SCD) or sickle cell anaemia is a haemoglobin-related inherited blood disorder resulting from a point mutation in the beta-globin gene (HBB) that causes the formation of aberrant sickle haemoglobin (HbS) protein, which makes the red blood cells (RBCs) stiff and sickle-shaped, which can obstruct tiny blood vessels, resulting in discomfort, organ damage and other issues (Kavanagh et al., 2022). CRISPR editing of mutations in HBB gene in hematopoietic stem and progenitor cells (HSPCs) resulted in eventual restoration of normal haemoglobin activity in the blood cells of patients (Lattanzi et al., 2021). In vitro, there was no noticed significant cytotoxicity of the CRISPR-Cas9, and more than 18 % genetic modification was examined in CD34+ cells. Additionally, the study conducted by Germino-Watnick et al. (2022) proved the successfulness of CRISPR in amending CD34+ HSPCs of SC anaemia patients taken out of the bone marrow.

Beta-thalassemia is a hereditary type of haematologic disorder caused by low-level production of beta-globin polypeptides in haemoglobin, the most important oxygen-carrying protein in the red blood cells. The illness is hereditary and caused by the HBB gene mutations (Lee et al., 2021). CRISPR/Cas 9 was used to treat a splice mutation IVS II-1 (G>A) in HBB that interrupts the splicing reaction through which pre-mRNA is processed to synthesise beta-globin (Servatian et al., 2023).

The Casgevy is a gene-modified therapy involving the use of the genome-editing system CRISPR-Cas9 as a major tool in the treatment of transfusion-dependent beta-thalassemia (TDT) and sickle-cell diseases (SCD) in patients of the age of twelve years and older (Kerwash et al., 2024). In addition to these, CRISPR could also be used to correct haemophilia, which is an X-linked, rare disorder, inheritable in nature, and elicited by the abnormal coagulation due to the insufficiency of clotting factors. Haemophilia B is related to all the characteristics of haemophilia with a conditional inactivation of the F9 gene that encodes a particular blood-clotting factor, coagulation factor IX (FIX), compared to haemophilia A, which is subject to a lack of factor VIII. Regeneron, in partnership with Intellia Therapeutics, is also developing CRISPR-based disease-curing options, either by knocking out the gene that causes disease or introducing a functional copy of the F9 gene to enable it to manufacture endogenous FIX. At the same time, both businesses are also working on a CRISPR-based treatment method for haemophilia A (Regeneron, 2020).

Fanconi Anaemia (FA) is a rare autosomal recessive familial blood disorder, which is characterised by damage to the bone marrow leading to aplastic anaemia, congenital abnormalities, and predisposition to

oncological complications. FA genes are a family of genes whose main roles are the repair of DNA and defence against DNA damage. Changes in this group of genes may result in genomic DNA damage as well as the increased risk of developing hematopoietic diseases, including bone marrow failure (BMF) and multiple myeloma. FA disease is caused by mutations in the FA gene locus that are inherited (Helbling-Leclerc et al., 2021). The targeting of FA gene by CRISPR-based base editing Homologous-directed repair (HDR) is one such therapy mode, which has the potential of correcting the double-strand DNA breaks (DSBs) and, in effect, restoring the endogenous DNA repair mechanism of the FA gene (Siegner et al., 2022). HDR-mediated CRISPR has been shown to have a therapeutic effect in the treatment of BMF, where it was shown to edit mutations in the genes of the FA complementation group F (FANCF) and group I (FANCI), which had corrected the abnormal sequence in patient-derived induced pluripotent stem cells (iPSCs) that were produced using the autologous fibroblasts (Martínez-Balsalobre et al., 2023).

**Eye-related Disorders**

Retinitis pigmentosa (RP), or inherited retinal dystrophy, is a group of inherited retinal diseases (IRD) with progressive loss of photoreceptor cells and the eventual restriction of sight. Over 71 genes (RP1, rhodopsin (RHO) and RP GTPase regulator (RPGR) among others) have been found to be associated with non-syndromic RP (Baltaziak et al., 2024). CRISPR/Cas9 treatment proved to be effective in halting the degradation of the retina in RP. Choi et al. (2023) reported the treatment of a mouse model of the disease by means of subretinal administration of gRNA-Cas9 plasmid, which successfully corrected the Rho S334 mutation, a common RHO gene variant in the RP severe form. Also, Meng et al. (2024) utilised CRISPR to enhance visual functions in vivo in rats using homology-independent targeted insertion (HITI), a strategy that can be utilised to effectively edit genes in post-mitotic cells.

Cataract is a pathological state in which a haze forms in the lens of the eye, which eventually results in reduced vision (Kholmatova, 2024). Various genetic mutations in different loci are responsible for the pathogenesis of congenital cataracts, with mutations on the alpha A-crystallin gene (CRYAA) on chromosome 21 having a major role to play. The CRYAA gene codes for CRYAA protein that prevents the aggregation of proteins within the cell, thus preventing cataracts. Besides that, the protein CRYAA is required to keep the lens transparent and with the proper optical focus (Hafizi et al., 2021). The other genetic determinant of congenital cataracts is the gap junction protein alpha 8 (GJA8) gene that encodes the connexin 50 (Cx50), which is a membrane protein also involved in the transparency of the lens as well as in the fibre cell proliferation (Shen et al., 2023). Whilst trying to understand the pathophysiology of congenital cataracts and assessing the efficacy of a new therapeutic agent, researchers have developed a rabbit model using the CRISPR-based gene knockout of GJA8 to reproduce the human pathology of congenital cataracts and offer an in vivo preclinical model in which a new cataract-prevention and treatment strategy could be tested (Sun et al., 2023).

New studies have shown that partial auditory recovery is feasible through the genetic restoration of the ATP2B2 and TMC1 genes (Tao et al., 2023).

**Table 1:** CRISPR-Cas9 and its potential therapeutic targeted genes for disease treatment

| Disease | Type | Mutated Genes | Role of Normal Genes | Mutation Effect | CRISPR Intervention | References |
|---|---|---|---|---|---|---|
| Cancer | Osteosarcoma | Cyclin-dependent kinase (CDK) 11 | Regulate cell cycle and other cellular processes | Can lead to tumorigenesis | Silence CDK11 gene | Zhra et al. (2025) |
| | Triple-negative breast cancer (TNBC) | CDK 7 gene | Regulate gene transcription | Disrupt cell cycle control and transcription | Silence CDK7 gene | Tiwari et al. (2023) |
| | Breast cancer | SHC SH-2-domain binding protein-1 (SHCBP1) | Regulate cell proliferation | Disrupting signalling pathways crucial for cell proliferation | Removal of SHCBP1 induces apoptosis in breast cancer cells | Sharma et al. (2021) |
| | Nasopharyngeal carcinoma (NPC) | Kelch domain containing 4 gene (KLHDC4) | Regulate protein degradation and influence signalling pathway in cancer | Mutations are related to cancer development and progression | Knockout of KLHDC4 retarded tumour cell migration and growth | Kanbar et al. (2024) |
| | Seen in many types of cancers | Multidrug resistance1 (MDR1) gene | codes for P-glycoprotein (Pgp) that acts as a multidrug efflux pump | Upregulation leads to chemotherapeutic drug resistance | Helped restore cells' sensitivity to chemotherapy in osteosarcoma cells | Vaghari-Tabari et al. (2022) |
| | Chronic | MUC18 | Encodes | Aggravate | Removal | Orr |

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|---|---|---|---|---|---|---|
| Allergy | obstructive pulmonary disease (COPD) and asthma | gene/melanoma cell adhesion molecule (MCAM) | for cell surface glycoprotein | inflammation and airway damage | of MUC18 gene could cause a significant reduction in interleukin levels (IL-8) | (2023) |
| | Severe combined immunodeficiency disease (SCID) | Janus kinase 3 gene (JAK3) | Plays role in cytokine signalling and immune response in the development of T cells and NK cells | can cause severe combined immunodeficiency disease (SCID) | restore faulty mutations in T cells | Önal (2024) |
| Immunological disorders | X-linked hyper-immunoglobulin M (IgM) syndrome | CD40LG gene | T-cells and B-cells activation and interaction | B cells face difficulty in transitioning from making IgM to other Ig classes | Restore T-cells' interaction with B-cells, which leads to class switching | Rogers & Cannon (2021) |
| Cardiovascular Diseases | Hypercholesterolemia | Proprotein convertase subtilisin/kexin type 9 (PCSK9) | Regulate cholesterol homeostasis | Overexpression leads to increased levels of LDL-C, which elevates the risk for CVDs, CAD and hypercholesterolemia | Treat CVD/genetic lipid disorders in the zebrafish model | Angom et al. (2024) |
| | Human immunodeficiency virus | Tripartite motif-containing | Targets the viral capsid | Alter the host's antiviral response and | Develop anti-HIV therapies | Somers (2023) |

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|---|---|---|---|---|---|---|
| Viral infections | (HIV) | protein 5 alpha (TRIM5α) gene | | contribute to the progression of HIV-1 | by enhancing the expression of TRIM5α | |
| | Human papillomavirus (HPV) | HPV E6 and E7 genes | N/A | Disrupt the normal functioning of the tumour suppressor proteins | Cas9 nuclease utilises a gRNA to target and cleave HPV DNA to inactivate the viral oncogenes | Kermanshahi et al. (2025) |
| | Epstein-Barr virus (EBV) | Ephrin receptor Tyrosine kinase A2 (EphA2) | Plays role in cell migration and proliferation | Causes infectious mononucleosis | CRISPR was used to modify the EBV genome in human cells by disrupting specific targeted regions in the BART promoter gene | Najafi et al. (2022) |
| Autosomal recessive disorder | Cystic fibrosis (CF) | Cystic fibrosis transmembrane conductance regulator (CFTR) protein/gene | Regulate normal movement of chloride and water across the cell membrane | Protein fails to carry out its normal function, leading to CF symptoms | CRISPR-based adenine base-editing was used to repair CFTR without causing off-target effects | Geurts et al. (2020) |
| X-linked disorder | Duchenne muscular dystrophy | DMD/dystrophin gene | Helpful for normal | Results in the gradual weakening | CRISPR was used by | Zhang et al. (2022) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | (DMD) | | muscle functioning by protecting muscle fibres from damage | and degeneration of skeletal muscles over time | utilising self-complementary adeno-associated virus (ScAAV) as a delivery vehicle | |
| Autosomal dominant disorder | Huntington's disease | Huntingtin/HTT gene | Play a role in normal development and nerve function | Presents a triad of psychiatric, cognitive and motor symptoms | CRISPR was used by pairing it with the nickase enzyme to treat Huntington's disease | Dabrowska et al. (2018) |
| Metabolic disorders | Hereditary tyrosinemia (HT) | Fumarylacetoacetate hydrolase (FAH) enzyme/FAH gene | Vital for tyrosine metabolism | Can cause hepatic cirrhosis, liver failure, hepatic cancer and kidney damage | CRISPR was used to repair FAH gene in an HTI mouse model | Urban et al. (2025) |
| | Hunter syndrome | Iduronate-2-sulfatase (IDS) enzyme/IDS gene | Disintegrate large sugar molecules | Causes the buildup of large sugar molecules that leads to progressive multisystemic disease | Used zinc finger nucleases (ZFNs) via an adeno-associated virus (AAV) vector | Li et al. (2020) |
| | Sickle cell (SC) anaemia | Beta-globin gene (HBB) | Helps in making the beta-globin protein | Formation of abnormal haemoglobin | CRISPR was employed to restore HBB function in hematopoi | Lattanzi et al. (2021) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Blood disorders | | | | | etic stem and progenitor cells (HSPCs) | |
| | Beta-thalassemia | HBB gene | Provides instructions for making the beta-globin protein of haemoglobin | Causes the deficiency of beta-globin chains in haemoglobin | CRISPR corrected the beta-thalassemia splice mutation (IVS-II-1G>A) in the HBB | Servatian et al. (2023) |
| | Haemophilia B | F9 gene | Prevent excessive bleeding by making blood clots | Impair the function of coagulation factor IX (FIX) | Therapeutics are developing CRISPR therapies to remove or replace F9 gene | Regeneron, 2020 |
| | Fanconi Anaemia (FA) | FA genes | Involved in DNA repair and protection | DNA damage and increased risk of bone marrow failure (BMF) and multiple myeloma | CRISPR-based base editing homology-directed repair (HDR) in FA gene can correct double-strand DNA lesions | Siegner et al. (2022) |
| Eye-related Disorders | Retinitis pigmentosa (RP) | RP1, rhodopsin (RHO) and RP GTPase regulator (RPGR) | Ensure healthy vision | Cause vision loss | CRISPR/Cas9 can help prevent retinal degradation by correcting | Choi et al. (2023) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | the Rho S334 mutation | |
| | Cataract | Gap junction protein alpha 8 (GJA8) gene | Ensure lens transparency and fibre cell growth | Cause blurry or hazy vision | A rabbit model has been developed by knocking out GJA8 gene using CRISPR-Cas9 to test new drugs | Sun et al. (2023) |

**The future of CRISPR gene editing tool:**

CRISPR has promising effects in future; it encompasses the fields of human disease treatment, medicine, agriculture, human health and basic biology. CRISPR has been proven to treat hearing and vision loss by replacing the mutated genes in the DNA with functional genes (Yin et al., 2023). But the CRISPR and its various effects have not been studied fully, and many risks are still unknown. Researchers and therapeutics are apprehensive about the probable off-target consequences and immune responses to the CRISPR therapies, as well as their ethical concerns (Aljabali et al., 2024). With the advancement in the field of genetics and medicine, scientists are passionate about improving the effectiveness and safety profile of these therapies. Moreover, with the penetration of AI in the field of medicine and surgery, the research in CRISPR is bound to expand (Dara et al., 2024). The future is very positive about the use of CRISPR in treating hereditary diseases and even targeting genes associated with psychological disorders (Gutiérrez-Rodríguez et al., 2023).

**Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

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