

Original Article

CRISPR-Cas Systems in Precision Medicine: Advances, Challenges, and Future Perspectives

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ABSTRACT: *The development of precision medicine was greatly helped by the CRISPR-Cas systems' strong ability to edit DNA. They make it simpler and more precise to correct DNA mistakes, which can help treat genetic disorders, cancer, and infections. The article compiles current information on CRISPR-Cas in precision medicine, covering its uses, recent achievements, and the issues that still need to be solved. We also consider what CRISPR-Cas systems may do in the future, such as their ability to aid personalized medicine and the ethical issues that require attention.*

KEYWORDS: *CRISPR-Cas, Personalized medicine, Gene drive technology, Synthetic biology, Genome editing, Genetic disorders, Cancer therapy, Antiviral therapy, Gene therapy, Ethical considerations.*

1. INTRODUCTION

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas (CRISPR-associated) systems have completely changed biotechnology and medicine by letting researchers reach parts that could not be reached before. Back when scientists first discovered them, bacteria and archaea used CRISPR-Cas systems as an effective protection against viruses. [1-3] When viral DNA is embedded into the bacteria's genome, it creates a record that helps the bacteria spot the virus and act against it if it enters the cell again. By using this method, scientists have developed new methods to manage genome editing in various living organisms, including humans, plants, and animals. CRISPR-Cas9, the leading form, has a guide RNA (gRNA) that guides the Cas9 enzyme to a specific part of the genome. With the Cas9 enzyme located at the target DNA site, researchers are able to add, delete or change genetic sequences more accurately than ever before. Precision medicine, which focuses on tailoring treatment to what is unique about each person, benefits greatly from this capability. Using genetic, environmental and lifestyle traits, precision medicine helps create effective treatments with fewer side effects than usual treatments applied to everyone.

In human health, using CRISPR-Cas technology, a number of genetic diseases can be treated by addressing the genetic changes related to sickle cell anemia, cystic fibrosis and some cancers. Furthermore, it is possible to use it to customize cancer treatments by genetically altering a patient's immune cells so they are more able to target cancer cells. CRISPR-Cas technology is being investigated in the fields of agriculture by making crops more resistant to diseases and stressors, and in ecology for the control of invasive species and to aid the restoration of endangered areas. Because these systems are precise and can be used in various ways, research has advanced and has also led to new conversations about how to use this technology properly. With the growth of this field, introducing CRISPR into clinical and biotechnological practices is expected to revolutionise the ways we study and manage biological systems, making medical treatments and advances in biotechnology both more effective and more tailored to each patient.

2. APPLICATIONS OF CRISPR-CAS SYSTEMS IN PRECISION MEDICINE

CRISPR-Cas technology has brought about major changes in precision medicine by permitting precise fixes to the genome that benefit many patients with different diseases. [4-7] The exact editing of DNA sequences lets scientists address genetic mutations, alter gene activity and adjust the immune system's actions. They are applied to genetics, cancer disease and treatment of infectious illnesses, among others. These applications are examined in more detail in the sections that follow.

2.1. GENETIC DISORDERS

The abnormal functioning of vital genes often results in specific genetic disorders that cause major physical and developmental problems. Using CRISPR-Cas systems, treatment is possible by correcting the genetic mutations involved in these disorders. The next section shows how CRISPR-Cas has made a difference in treating genetic disorders.

2.1.1. SICKLE CELL ANEMIA

Sickle cell anemia is an inherited blood problem caused by a change in the β -globin gene that produces misshapen haemoglobin. Consequently, red blood cells become twisted in a sickle-like way, which results in blockages, intense pain and problems for organs. Using CRISPR-Cas9, researchers have successfully corrected the sickle cell mutation in stem cells taken from patients with the condition. A research group used HSCs to edit the β -globin gene and return the changed cells to patients

through transplant. Following treatment, the disease symptoms in patients declined, their pain episodes were less frequent, and their haemoglobin levels increased. Due to its success, CRISPR-Cas is being developed for use in treating sickle cell anaemia and other genetic blood disorders.

2.1.2. CYSTIC FIBROSIS

Cystic fibrosis is a dangerous, genetically based condition caused by changes in the CFTR gene. The change in the gene affects how cells transport chloride, causing fluid in the lungs, pancreas and other organs to turn thick and sticky. Laboratory research with CRISPR-Cas9 has shown that CFTR mutations in lung epithelial cells, the cells that are mainly involved, have been corrected. A study of CRISPR-edited lung cells found that chloride ion transport increased, and mucus production diminished, suggesting gene editing could help patients with cystic fibrosis for a long time. Although their applications in the clinic are not yet ready, CRISPR-based therapies have the potential to tackle the main issue behind the disease.

2.2. CANCER

Genetic mutations cause the out-of-control growth of cells that happens in cancer. The CRISPR-Cas systems allow scientists to remove or reduce harmful mutations, stop new tumors from appearing and boost the way the body defends against cancer cells on its own. The applications below demonstrate how CRISPR is utilised in cancer treatment.

2.2.1. TARGETING ONCOGENES

When an oncogene is either mutated or expressed too much, it causes cells to multiply and spread, which helps cancer grow. In different types of cancer cells, CRISPR-Cas9 has been used to target and disarm oncogenes. As an example, research teams have managed to block the MYC oncogene, which is seen in many cancers, resulting in smaller cancers and better survival for animals tested. Using this method of gene editing helps directly turn off cancer-related genes and could improve the results of cancer treatment.

2.2.2. ENHANCING IMMUNE RESPONSE

CRISPR-Cas9 may enable the immune system to attack tumours with greater force. A promising strategy is to modify T cells, whose job is to spot and attack cancer cells. Using CRISPR-Cas to make T cells carry CARs aimed at cancer cell proteins boosts the success of immunotherapy. In clinical trials, patients with aggressive cancers were treated with CRISPR-modified CAR-T cell therapy, resulting in their tumours shrinking and improved survival. CRISPR has greatly improved the way cancer treatment is personalized, as it lets doctors make exact genetic changes to boost the immune response.

2.3. INFECTIOUS DISEASES

CRISPR-Cas systems can address infections by attacking bacteria, viruses or improving the immune system's response. The sections that follow explore how CRISPR is used in treating viral and bacterial infections.

2.3.1. ANTIVIRAL THERAPY

Researchers are considering CRISPR-Cas9 as a strategy to prevent replication and illness from viruses by disrupting their genetic code. CRISPR has been applied to treat HIV-1 by helping experts remove bits of viral DNA inserted into cells, thus ending dormant forms of the infection. CRISPR has also been used on the Hepatitis B virus (HBV) and Human Papillomavirus (HPV), demonstrating that it helps reduce viral quantities and may prevent disease recurrence. The results demonstrate that using CRISPR in antiviral therapies could be a major advancement for treating persistent viral infections.

2.3.2. ANTIBACTERIAL THERAPY

The development of bacteria resistant to modern medicine is a major problem for people everywhere. They could solve this problem by focusing on antibiotic resistance genes in bacteria and allowing conventional antibiotics to be used again. Therapies based on bacteriophage have been created with CRISPR so that they can eliminate drug-resistant strains in bacteria. Additionally, using CRISPR, essential bacterial genes have been targeted for elimination, resulting in the destruction of bacteria and the avoidance of infections. These methods enable the development of CRISPR-based treatments for bacterial infections that might fight antibiotic resistance.

Genome editing has been greatly improved by CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems, because they make it possible to change DNA sequences with great specificity. In this image, researchers have organized the main CRISPR-Cas genome editing methods into four large groups: DSB editing, base editing, prime editing and epigenome editing. The result of these methods is that genetic engineers can make more precise changes in DNA, which they use in medical treatments and research.

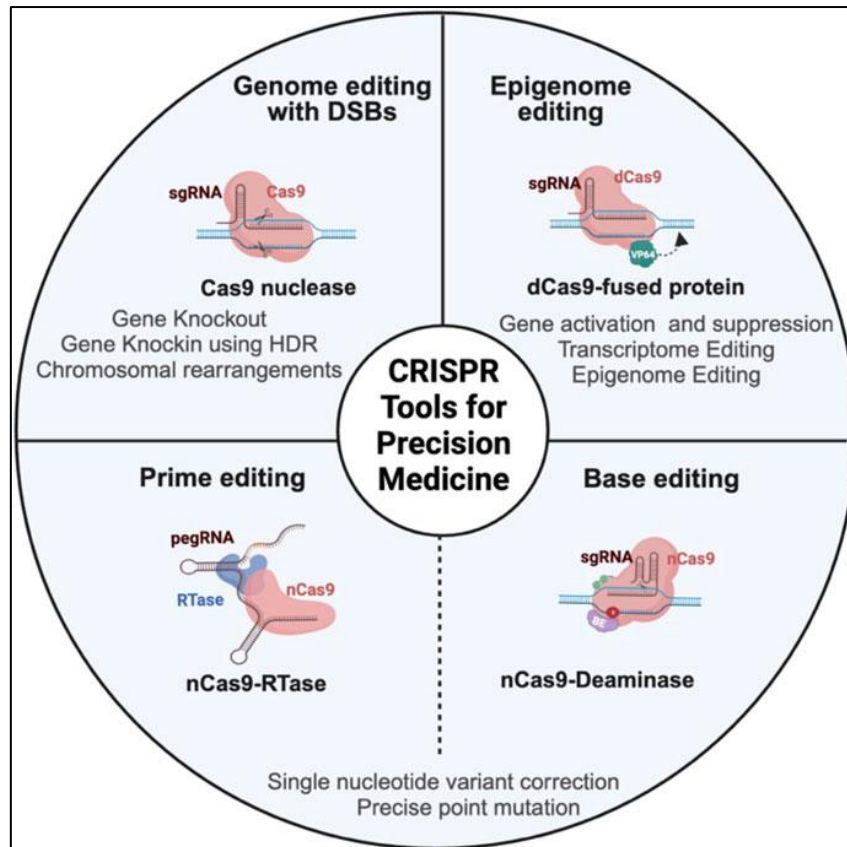


FIGURE 1 CRISPR tools precision medicine

A particular approach focuses on shaping DNA with double-strand breaks using Cas9 nuclease. The method is based on Cas proteins from CRISPR, which allow scientists to create cuts in the DNA, resulting in gene knockout, gene insertion using HDR and chromosome reordering. Off-target problems and risks to the genome are common when viral vectors are used in gene therapy. Another tool, called prime editing, accurately changes DNA without breaking the genome. A specific change in DNA is introduced by pairing Cas9, a single-guide RNA and RTase. Single nucleotides can be corrected, and tiny insertions and deletions can be made; it's unlikely to cause unneeded mutations, making this technique effective for treating genetic disorders. The technique is different from others because it modifies a single DNA base without splitting the entire DNA strand. The process utilises an altered Cas9 protein (nCas9) linked to a deaminase enzyme, allowing for the specific modification of nucleotides. This technique works very well for correcting gene mutations connected to genetic diseases and is considered more precise than traditional CRISPR-Cas9. Using epigenome editing, a catalytically blocked Cas9 (dCas9) joined with regulatory proteins is used to regulate genes without changing the DNA's code. Because it allows genes to be switched on or off, it's an important tool for learning about gene function, handling health issues connected to incorrect gene activity and creating chemical compounds for epigenetic therapy.

3. RECENT ADVANCES IN CRISPR-CAS TECHNOLOGY

The technology behind CRISPR-Cas is advancing quickly, bringing added accuracy, performance and uses to genome editing. Although traditional CRISPR-Cas9 is powerful, it still faces some difficulties, including the potential to hit the wrong areas in the DNA and break it into two pieces. [8-12] Advances in base editing, prime editing, CRISPR-Cas13 and CRISPR-Cas12 are working to address these problems and increase the use of precision medicine, diagnostics and therapeutic operations. The application of CRISPR-Cas to precision medicine is described, showing the steps required for genome editing. In the beginning, Target Identification requires researchers to find out exactly which gene sequence must be modified. When the target is chosen, it is then important to direct a guide RNA (gRNA) to the wanted spot on the DNA using local Guide RNA Design. After that, the proper Cas protein is picked through Cas Enzyme Selection based on the needs of the experiment. During the Delivery System stage, the goal is to get the CRISPR components to the right cells. Cancer vaccines can be delivered using viral vectors, lipid nanoparticles or by electroporation. In the cell, Genome Editing is carried out, which means DNA can be changed by inserting, deleting or correcting parts of its code. After the changes have been made, Validity and Testing are performed to make sure the modifications are effective and improve the targeting system without causing issues elsewhere. In Clinical Applications, cells or organisms are tested and put to use, primarily for treating inherited diseases, developing personalised patient treatments, or advancing research in biomedical fields.

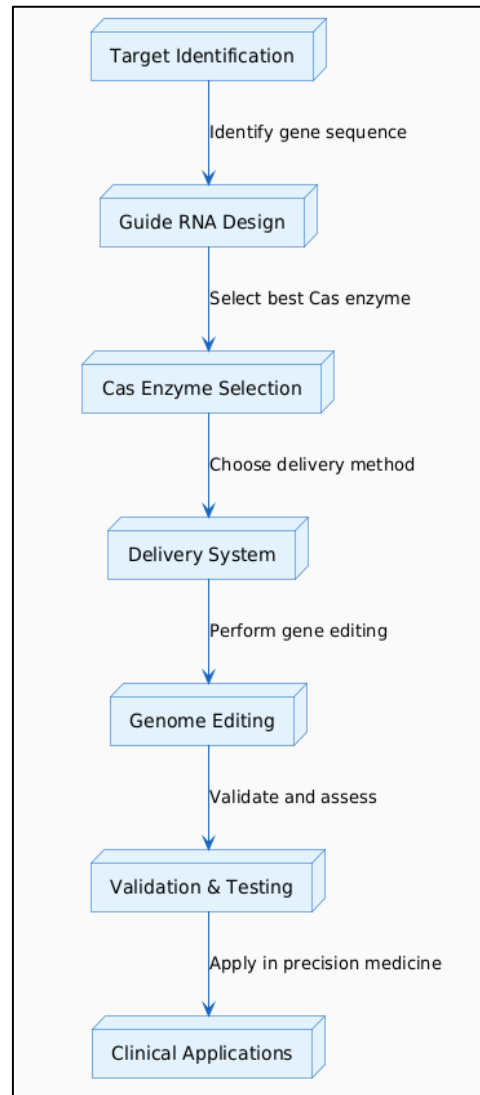


FIGURE 2 CRISPR-Cas workflow in precision medicine

3.1. BASE EDITING

Base editing is a new method using CRISPR-Cas that lets scientists directly switch one base in DNA for another without breaking both strands. In contrast to traditional CRISPR-Cas9, which uses the cell's repair process and can occasionally lead to side effects, base editing uses a different type of Cas9 fused to either cytidine deaminase or adenosine deaminase. Specific nucleotides are the only ones that are changed by the system, lowering the chance of mistakes in the DNA.

Scientists have used base editing to address point mutations which cause genetic diseases. Genetic therapy has been applied to treat sickle cell anemia and beta-thalassemia, which happen because of a small change in the genes for haemoglobin. Base editing, which replaces harmful mutations with harmless ones, is an encouraging new treatment approach. Because base editing is very specific, it causes fewer off-target changes than ordinary genome editing methods.

3.2. PRIME EDITING

Prime editing outperforms both old CRISPR-Cas9 genome editing and base editing in terms of precision and flexibility. Because you do not need double-strand breaks or donor DNA, this method becomes easier to use and more flexible. The technique utilises a Cas9 nickase linked to a reverse transcriptase enzyme, along with a prime editing guide RNA (pegRNA), to encode the desired change. It can accurately correct many genetic mutations, such as insertions, deletions, and changes in the four bases, with better results than other techniques. In the laboratory, prime editing has fixed genetic changes linked to Tay-Sachs disease, cystic fibrosis and sickle cell anemia. Its precision in editing the genome keeps it stable and more accurate, which is perfect for applications in precision medicine.

3.3. CRISPR-CAS13

CRISPR-Cas13, unlike most CRISPR systems, targets RNA rather than the DNA of the cell. Its special quality allows it to be used effectively in RNA-related diagnostics, gene control and fighting viruses.

CRISPR-Cas13 is especially effective in removing viral RNA and is being tested as a way to treat RNA viruses like SARS-CoV-2 (COVID-19), influenza and Zika. Cas13 uses targeted cuts of the viral RNA to stop the virus from copying itself and lowers the impact of an infection. Scientists have also applied CRISPR-Cas13 to manage gene activity by controlling mRNA in the cell, which helps them to study how genes work and create RNA therapies for neurological diseases, cancers and metabolic problems. In SHERLOCK (Specific High-Sensitivity Enzymatic Reporter Unlocking) technology, CRISPR-Cas13 is used to quickly and accurately detect low concentrations of viral RNA. As a result, Cas13 is very useful for spotting signs of disease early and monitoring pathogens throughout the process.

3.4. CRISPR-CAS12

CRISPR-Cas12, a member of the Type V CRISPR-Cas family, is known for its use in DNA detection, diagnostics, and genome editing. Cas12a (Cpf1) needs no adjacent PAM sequence to target a gene, so it is simpler to use for genetic modifications than Cas9.

CRISPR-Cas12 has found useful applications in disease diagnostics. DETECTR, using Cas12, has been invented to detect viral and bacterial DNA rapidly and with great accuracy. So, Cas12-based methods have helped in the quick detection of both human papillomavirus (HPV) and tuberculosis, replacing traditional testing with a method that is faster, less costly and just as accurate. In addition to being used for disease detection, CRISPR-Cas12 is under study for gene editing, with some scientists finding that it can be more specific and efficient than Cas9 in certain applications. Cas12 can introduce precise cuts instead of jagged cuts, which allows scientists to add or remove genes with greater precision.

TABLE 1 Comparison of CRISPR-Cas systems

CRISPR-Cas System	Target	Key Features	Applications
CRISPR-Cas9	DNA	High efficiency, widely used	Genetic disorders, cancer, and infectious diseases
CRISPR-Cas13	RNA	RNA targeting, antiviral therapy	Antiviral therapy, gene regulation
CRISPR-Cas12	DNA	High specificity, DNA detection	Diagnostics, genome editing
Base Editing	DNA	No DSB, high precision	Genetic disorders, cancer
Prime Editing	DNA	Flexible editing, no DSB	Genetic disorders, cancer

4. CHALLENGES AND LIMITATIONS

Although CRISPR-Cas research is advancing rapidly, some obstacles and limitations must be addressed first for the technology to be fully utilised in precision medicine. Issues in CRISPR-based therapies arise from challenges in targeting the procedure, delivering the treatment to the intended cells, and meeting ethical and regulatory standards. Addressing these flaws is crucial for CRISPR to be widely adopted in medicine.

4.1. OFF-TARGET EFFECTS

A major worry about CRISPR-Cas genome editing is that the Cas enzyme might cut DNA at a site it wasn't supposed to, an issue called off-target effects. These additional changes may result in unplanned genetic alterations in cells, which can cause cells to malfunction, develop cancerous mutations, or trigger an immune system response. Many obstacles to applying CRISPR in people, specifically for gene therapy, are caused by these risks.

Scientists have developed more accurate variants of Cas9, such as SpCas9-HF1 (high-fidelity Cas9) and eSpCas9 (enhanced specificity Cas9), to minimise accidental genetic modifications. Shorter gRNAs, known as truncated gRNAs, have been demonstrated to improve specificity and lower the possibility of off-target damage. Genome editing experiments become safer and more accurate when tools and deep learning algorithms predict and analyze the possible effects on unintended DNA before starting work. Advances like these need more research before they can control which cells are edited and ensure the results are not damaged.

4.2. DELIVERY METHODS

To use CRISPR-Cas in therapy, it must be possible to introduce the Cas enzymes and guide RNAs to the target cells with safety and efficiency. After all, creating systems that effectively deliver genome editors continues to be a major challenge. Some of the traditional methods for delivering genetic medicine, including viral vectors and electroporation, have issues with efficiency, safety, and the way they trigger an immune system response. Viral vectors may correctly edit the genome, but they can cause the immune system to notice, may be unsafe for the genome and handle only smaller genetic materials. While electroporation does well in laboratory settings, it can cause cell harm and cannot always be used for in vivo gene therapy.

To solve these issues, scientists are investigating improved delivery methods, one of which is:

- Nanoparticle-based delivery: LNPs (lipid nanoparticles) are used in mRNA vaccines and have been adopted to transport CRISPR into cells. With LNPs, it becomes possible to efficiently put CRISPR components into certain cells, preventing immune problems and making the treatment less toxic.
- Ribonucleoprotein (RNP) complexes: Pre-assembling Cas9 with its guide RNA into RNP complexes allows better precision and fewer off-target effects compared to plasmid or viral methods.
- Peptide-based and exosome-based delivery: The use of cell-penetrating peptides or natural extracellular vesicles as delivery systems is also effective because it leads to higher accuracy and doesn't cause much of an immune reaction.

4.3. ETHICAL AND REGULATORY CONSIDERATIONS

Many people are worried about using CRISPR-Cas technology in humans, especially because it may change the genes of future generations. While some of the genetic changes from somatic cell editing are lost, germline editing can be inherited, which raises a variety of ethical, social and moral issues. Serious issues that are discussed involve:

- Unintended consequences: Making improvements to the human genome could lead to untested consequences in future generations, including more genetic diseases or a decline in genetic diversity.
- Equity and accessibility: CRISPR-based therapies could create disparities in healthcare, where only the wealthy have access to genetic enhancements, leading to a new form of genetic inequality.
- Designer babies: Non-therapeutic genetic enhancements, which may give babies greater intelligence or athletic skills, spark discussions about their ethics and, sometimes, who should consent.

TABLE 2 Clinical Trials Using CRISPR-Cas Technology

Diseases	CRISPR-Cas System	Target	Status
Sickle Cell Anemia	CRISPR-Cas9	β -globin gene	Phase I/II
Cystic Fibrosis	CRISPR-Cas9	CFTR gene	Preclinical
Cancer (MYC)	CRISPR-Cas9	MYC oncogene	Preclinical
Cancer (CAR-T)	CRISPR-Cas9	T cells	Phase I
HIV-1	CRISPR-Cas9	HIV-1 provirus	Preclinical

5. FUTURE PERSPECTIVES

Continuous improvements in CRISPR-Cas technology are opening new doors for personalized medicine, gene drive systems and synthetic biology. These developments could transform how we treat diseases, steer new strategies for disease control and widen the reach of biotechnology. Even as these technologies grow, we must make sure to deal with their ethical, legal and environmental concerns to keep things safe and ethical.

5.1. PERSONALIZED MEDICINE

Using CRISPR-Cas technology in personalized medicine should help develop therapeutic solutions tailored to each patient's needs for genetic disorders, cancer and other diseases. Many traditional approaches offer the same advice for all, which may not align with the genetic differences of each patient. CRISPR-based medicine can therefore adjust the use of genome therapy to tackle genetic mutations that are personal to each patient. Those diagnosed with rare genetic conditions originating from a point mutation might benefit from CRISPR therapy that is customized to repair the faulty gene with less chance of side effects. CRISPR therapies in oncology help attack parts of cancer cells that carry specific mutations, leading to better treatment results with fewer harmful effects than common chemotherapy or radiation.

Integrating CRISPR-Cas systems with genomic sequencing provides a method to make predictions about diseases a person might develop. Analyzing a person's genes can help clinicians predict risks and treat them before the disease begins, making prevention the priority. With this method, there is the potential for better patient results, lower healthcare bills and a new way of practising medicine.

5.2. GENE DRIVE TECHNOLOGY

CRISPR-Cas-powered gene drive technology enables the quick introduction of traits into entire populations of organisms. With this technology, it will be easier to confront diseases like malaria, dengue and Zika, which are spread by way of mosquitoes and other insects. CRISPR-based gene drives let scientists edit the genes of mosquitoes to make them less likely to pass on diseases. Experts have changed the genes of mosquitoes so they resist getting infected with the parasite that causes malaria. As genetically modified mosquitoes reproduce, most of their offspring receive the edited DNA, helping the wanted trait to become common quickly. Vaccination can cut down on or even get rid of diseases that take the lives of millions of people each year. Genetics experts are concerned about the ethical, environmental and legal sides of gene drive technology. We do not yet completely know the impact of releasing genetically modified organisms into the environment. If we attempt to remove mosquitoes, some species that live on mosquitoes could be adversely affected. Some are worried that gene drives could escape control or be incorrectly used by others. Therefore, scientific and regulatory rules must exist to oversee, maintain, and

supervise the application of gene drive technology. For this technology to be applied safely and ethically, scientists, policymakers and environmental organizations must unite from different countries.

5.3. SYNTHETIC BIOLOGY

CRISPR-Cas technology is being integrated into synthetic biology to allow the creation of biological systems with unique functions. This field of Biology develops genetic circuits that detect, interpret and react to specific biological signals, resulting in new technologies found in medicine, biotechnology and environmental science.

CRISPR-Cas is employed in synthetic biology to create programmable circuits that support both diagnostics and therapeutics. As an example, scientists have built CRISPR-based sensors that let them spot pathogens or cancer biomarkers whenever they are present. They can be equipped with protective gear or mobile devices to quickly and accurately detect diseases in countries where resources are scarce. CRISPR-Cas technology is also being used to develop microorganisms for use in biomanufacturing. Bacteria and yeast have been updated by scientists to manufacture things, including biofuels, drugs and plastics, that are environmentally safe. Using CRISPR, scientists can make these microbes more efficient and sustainable, which supports making industry more environmentally friendly. CRISPR technology may be used in efforts to protect the environment.

An example is that researchers are examining if they can modify bacteria to turn plastic trash into something easy to recycle or neutralize harmful substances in the environment. By following this approach, we may find clever ways to tackle climate change and control pollution. Dealing with CRISPR-Cas technology in synthetic biology warrants looking at both ethical and safety issues together. Genetic risks and biosecurity threats should be looked at carefully, with the help of scientists, ethics and regulations.

6. CONCLUSION

CRISPR-Cas systems are now commonly used to edit genes, which is crucial for precision medicine. The rapidly and accurately changing genome allows for improved treatment of genetic disorders, cancer, and infectious diseases. Despite the progress, questions such as off-target impacts and how to deliver the methods and ethics need to be addressed before CRISPR-Cas can fulfil its potential. Prospects such as personalized medicine, gene drive technology and synthetic biology look very promising for CRISPR-Cas systems in precision medicine.

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