CRISPR-Based Therapeutics: Breakthroughs in Targeting Cancer

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***Abstract*—Cancer continues to be a significant economic and social burden and is the second greatest cause of mortality worldwide. It is brought on by the buildup of genetic and epigenetic changes in two different gene types, proto-oncogenes and tumor suppressor genes (TSGs). Even though our molecular understanding of cancer is still growing, additional work is required to create novel treatment instruments and strategies that make use of these developments. The consistent advancements in genome editing, particularly that which uses programmable nucleases and clustered regularly interspaced short palindromic repeats (CRISPR) to precisely alter genetic material, have opened up a plethora of opportunities to further biomedical research and improve human health. CRISPR/Cas9 systems-mediated gene editing is expected to develop into a useful therapeutic tool for the treatment of some malignancies and hereditary illnesses. The CRISPR-Cas9 system was first employed by bacteria to protect themselves from various bacteriophages. More recently, it has gained noteworthy recognition for its potential to treat genetic diseases and cancer. Another powerful technique for developing animal genetic models for the research and treatment of human genetic illnesses, especially those linked to point mutations, is CRISPR/Cas genome editing. In this review, we have emphasized the role of the CRISPR-Cas9 system in cancer treatment, along with its potential uses in different cancer types and cancer biology, as well as the significant obstacles that must be overcome before it can be clinically applied to a complex and multifactorial disease like cancer.**

***Index Terms*—Genome editing, CRISPR-Cas9, Tumour suppresser genes (TSGs), Cancer therapy, Genetic and epigenetic changes.**

History & Discovery of CRISPR:

Today, CRISPR is well-known as a precise gene editing tool, but it took many years for scientists to figure out what it was and how to harness its potential. Let's discuss the inventors of this gene editing tool, the scientists who championed this technology, and the history behind these breakthroughs.

While Doudna and Charpentier were the first to adapt CRISPR-Cas9 as a gene editing tool, the history goes back a little further than their 2012 publication. In 1993, Dr. Fransisco Mojica, a scientist at the University of Alicante in Spain, identified repetitive palindromic segments of DNA interspaced with other fragments of genetic material in bacterial genomes. Dr. Mojico gave these regions the name CRISPR, and proposed that they are a component of the bacterial immune system. In 2007, a team of scientists led by Dr. Philippe Horvath experimentally demonstrated Mojica's theory.

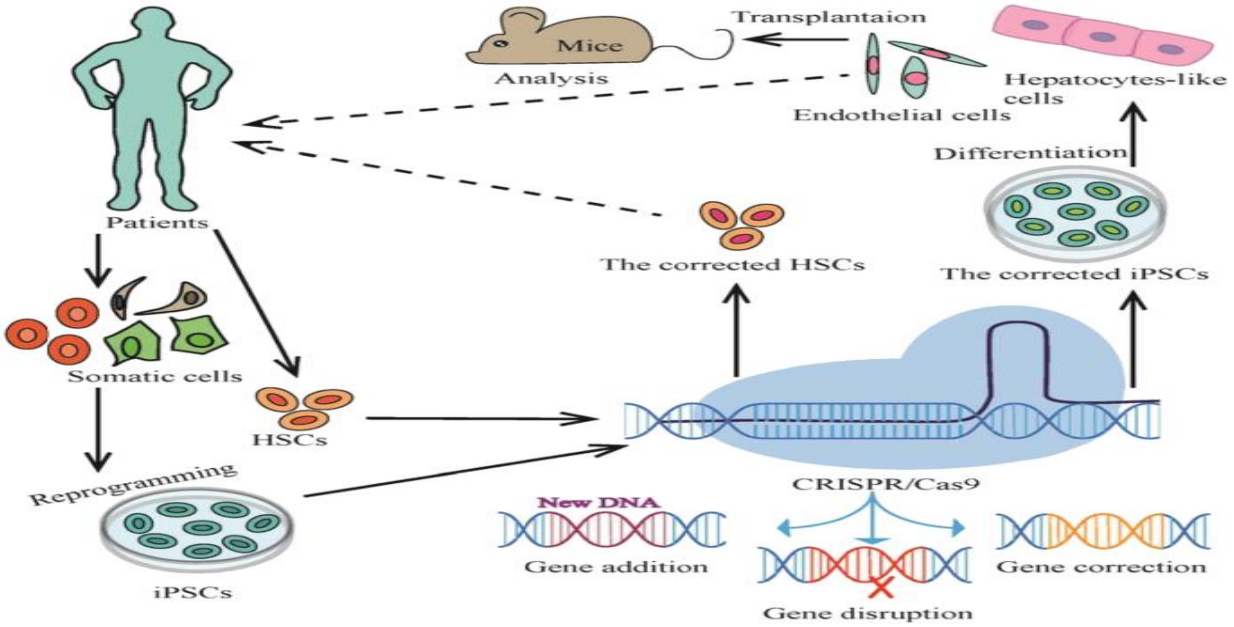
Since its adaptation by Dr. Doudna and Dr. Charpentier, this versatile gene editing technology has progressed rapidly. It has been adapted for many different purposes, including RNA editing, base and prime editing, live imaging, and diagnostics. It has been used to edit DNA in a variety of organisms, including humans.

In 2019, the first CRISPR clinical trials began, harvesting cells from patients with sickle cell disease (SCD) and editing them in vitro before infusing them back into the body - a method known as cell therapy. After the success of SCD cell therapy trials, a CRISPR treatment was injected directly into human patients for the first time in 2020. This technique is known as gene therapy, and was used to treat hereditary blindness.

I. INTRODUCTION

The use of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) in cancer therapy has the potential to revolutionize the way for treating different diseases. CRISPR technology allows for

precise and efficient manipulation of the genome, and its application in cancer research has the potential to target specific genetic mutations that drive the growth and spread of tumors. In recent years, there has been a growing body of research exploring the use of CRISPR-based gene editing in cancer therapy, with several preclinical studies and clinical trials demonstrating promising results. The discovery of CRISPR technology in 2012 marked a significant milestone in the field of genome editing illustrates the evolution of CRISPR tools used for exploring cancer biology. CRISPR-associated enzymes, such as Cas9, can be programmed to target specific DNA sequences, and when combined with guide RNAs, can be used to cut, modify or delete genes in a precise manner. This technology has been used in a wide range of applications, including basic research, gene therapy, and agriculture. However, its potential application in cancer research has attracted particular interest due to the ability to target the genetic mutations that drive the growth and spread of tumors. There are several different CRISPR-based strategies that have been proposed for cancer therapy. One approach is to inactivate genes that drive tumor growth. For example, using CRISPR to inactivate the oncogene MYC has been proposed as a way to halt tumor growth.



The MYC gene is known to be overactive in many types of cancer, and its inactivation could potentially slow down or stop the progression of the disease. Another approach is to enhance the immune response to cancer cells. For example, researchers have used CRISPR-based gene editing to knockout or decrease the expression of the PD-1 protein on T cells, which helps to improve their ability to target and kill cancer cells. Additionally, CRISPR-based gene editing can be used to repair genetic mutations that cause cancer, such as in the case of inherited forms of cancer caused by BRCA1 and BRCA2 mutations. For example, studies have shown that CRISPR-Cas9 can be utilized to correct BRCA1 mutations in human cells, demonstrating the potential for this technology in cancer therapy. Furthermore, CRISPR-based gene editing can also be employed in immunotherapeutic strategies for cancer treatment. For instance, T cells can be engineered using CRISPR to express receptors that specifically target tumor cells, enhancing the body's immune response against cancer. Preclinical studies and clinical trials have been conducted using these strategies, and they have demonstrated promising results. For example, inactivating the MYC oncogene in animal models of lymphoma has been shown to reduce tumor growth. Similarly, increasing the expression of PD-1 on T cells has been shown to enhance the ability of these cells to target and kill cancer cells in animal models. However, despite the promising results obtained in preclinical

studies, there are still many challenges that need to be overcome for CRISPR-based cancer therapy to become a viable clinical option. One of the main challenges is the risk of non-specific site effects, which can occur when CRISPR enzymes target unintended regions of the genome. Safety and delivery are also critical challenges that need to be addressed.

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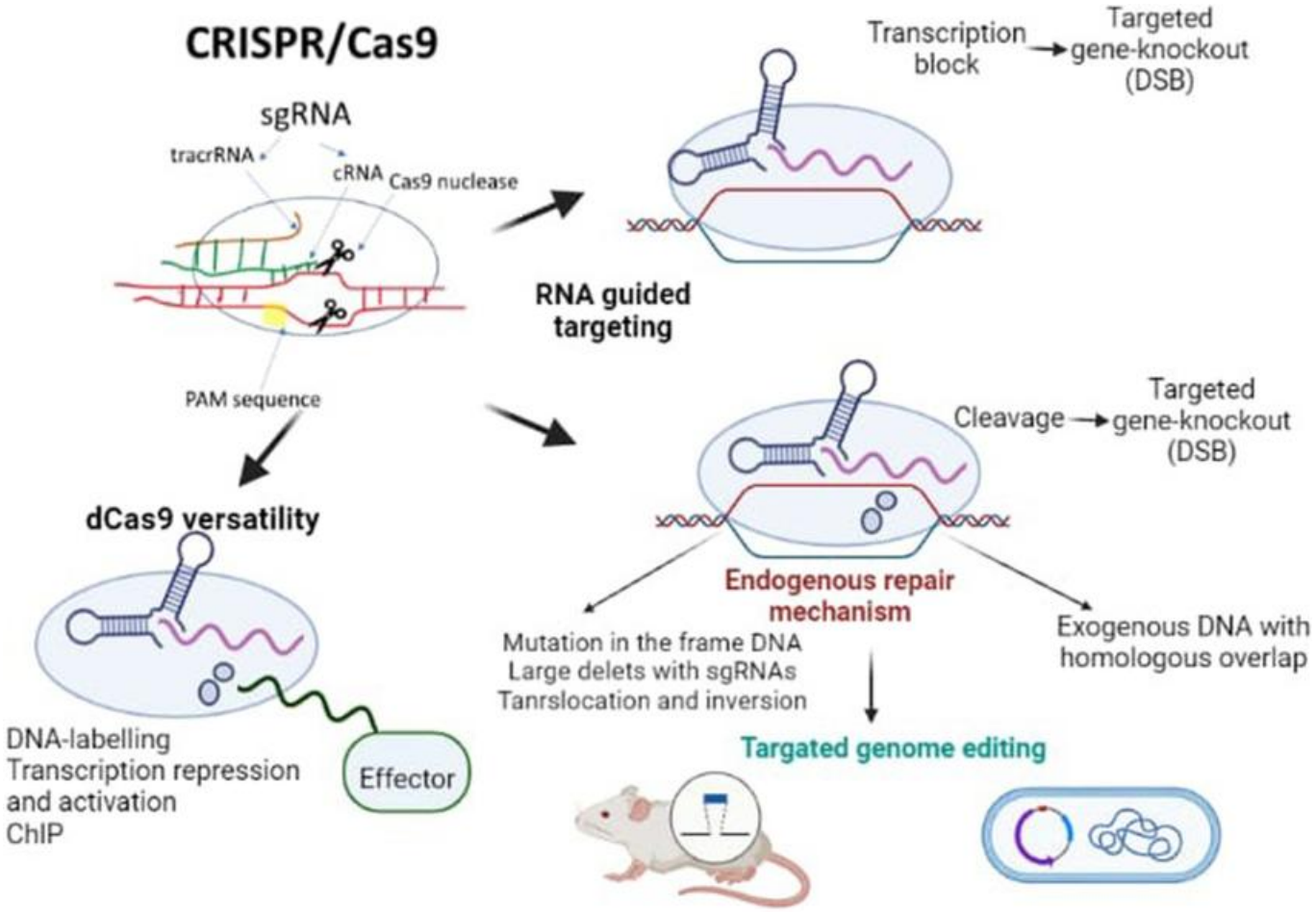
II. CRIPSR/CAS SYSTEM MECHANISM

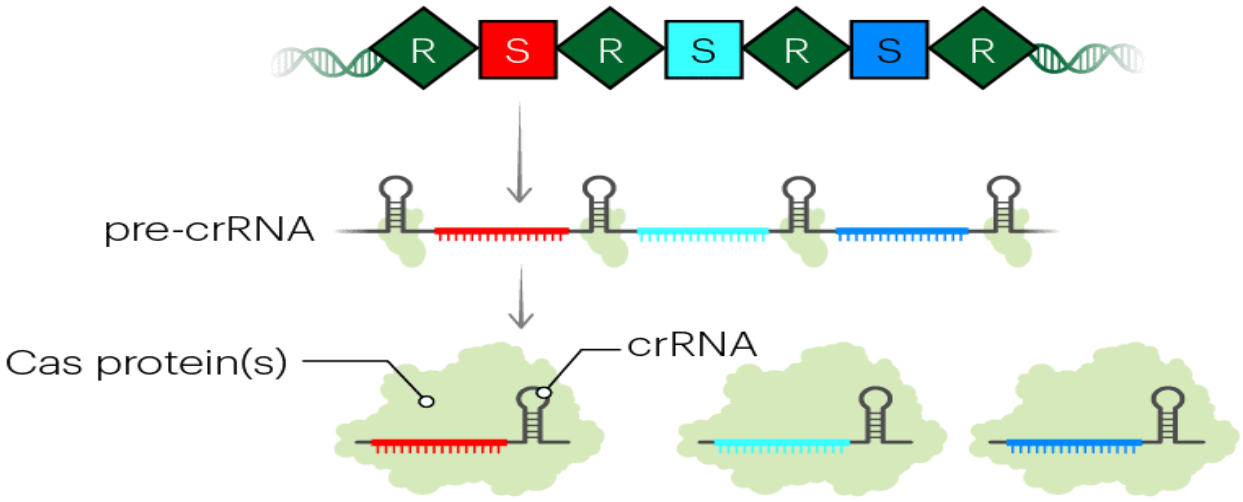
The CRISPR-Cas mechanism is a bacterial immune system that provides adaptive immunity against viruses by storing viral DNA fragments (spacers) in CRISPR arrays. Upon re-infection, these spacers are transcribed into crRNAs that guide Cas proteins (like Cas9) to the target viral DNA, where they induce a double-strand break and neutralize the invader. The targeted DNA can then be repaired, modified, or deleted by the host cell's machinery, enabling genetic engineering applications.



Spacer Acquisition:

Viral Attack:
When a bacterium is infected by a virus, a portion of the viral DNA is cut out.
Integration:
Specialized Cas proteins, such as Cas1 and Cas2, integrate this viral DNA segment (called a spacer) into the bacterium's CRISPR array, located on its own genome.
CRISPR Array:
The CRISPR array consists of short, repetitive palindromic sequences interspersed with these acquired viral spacers. This array acts as a genetic "memory" of past infections.

crRNA Biogenesis;



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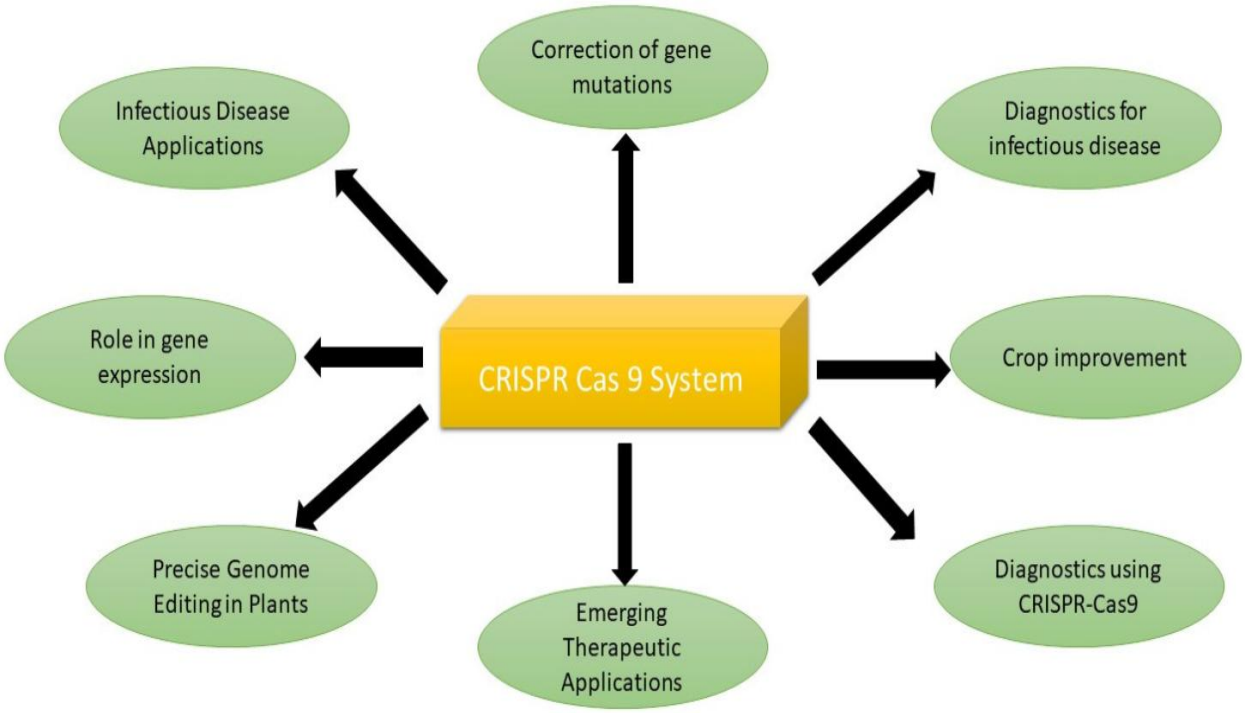
Transcription:
The integrated viral sequences in the CRISPR array are transcribed into a long RNA strand.
Processing:
This RNA molecule is then processed and cut by Cas proteins into shorter units called CRISPR RNAs (crRNAs). Each crRNA contains one spacer sequence and a portion of the repeat.

Key Applications:
CRISPR is a versatile genome editing technology with key applications in gene therapy for genetic diseases (like sickle cell anemia and transthyretin amyloidosis), cancer treatment (via T-cell modification and tumor suppressor gene activation), agriculture (improving crop traits like disease resistance), and creating disease models in animals to study genetics and develop drugs. The technology works by using a guide RNA to direct a Cas protein to a specific DNA site, where it creates a break that the cell's repair machinery then uses to make precise edits, such as correcting a mutation or adding new genetic material.



1. Gene Therapy:

- Correcting disease-causing mutations: CRISPR can be used to fix specific genetic defects that cause hereditary conditions like sickle cell anemia, Leber congenital amaurosis, and corneal dystrophy.
- In vivo editing: It can be delivered directly into the body (in vivo) to treat diseases by targeting cells with the faulty genes, such as in the treatment of transthyretin amyloidosis.

2. Cancer Treatment:

- Immunotherapy enhancement: CRISPR can be used to edit T cells to make them more effective at recognizing and attacking cancer cells, improving the outcomes of therapies like CAR-T cell therapy.
- Targeting tumor cells: The technology can be used to inactivate oncogenes (cancer-causing genes) or activate tumor suppressor genes to control the growth of cancer cells.
- Overcoming drug resistance: CRISPR can disrupt genes that make cancer cells resistant to chemotherapy, increasing their susceptibility to treatment.
- Early detection: CRISPR-based diagnostic tools can detect cancer-associated genetic mutations, aiding in early diagnosis.

3. Agriculture:

- Crop improvement: CRISPR can enhance crops by modifying genes to increase yield, improve nutritional content, and boost resistance to diseases and environmental stressors like drought.

4. Biotechnology and Research:

- Creating disease models: CRISPR allows for the efficient and cost-effective creation of genetically modified animal and cell models for human diseases, which are crucial for understanding disease pathogenesis and developing new drugs.
- Gene function studies: Scientists use CRISPR to knock out, activate, or repress genes to study their functions and their roles in various biological processes.

Evidence from Preclinical and Clinical Studies

Extensive preclinical research has demonstrated the therapeutic potential of CRISPR-based genome editing in various cancer models. Early in vitro studies successfully used CRISPR-Cas9 to knock out oncogenes such as *E6* in HPV-positive cervical cancer cell lines, resulting in Restoration of p53 activity and apoptosis. Similar approaches in colorectal and lung cancer cell lines targeting KRAS and TP53 have shown significant suppression of tumor cell proliferation. In vivo experiments further validated these findings, with CRISPR-mediated deletion of immune checkpoint genes such as PD-1 in mouse T cells leading to enhanced anti-tumor immunity in melanoma models. The successful translation of these findings into clinical settings is now underway. In 2016, China initiated the first human trial using CRISPR-edited T cells to disrupt *PD-1* in patients with refractory lung cancer, reporting good tolerability and modest tumor control. A landmark Phase I trial in the United States by Stadtmauer et al. (2020) demonstrated the safety of multiplex CRISPR editing in T cells targeting *PD-1* and *TCR* genes in patients with advanced myeloma and sarcoma. Delivery of CRISPR components through lipid nanoparticles has also shown promise; for instance, silencing of the *PLK1* gene in hepatocellular carcinoma-bearing mice led to over 70% reduction in tumor growth, highlighting the feasibility of non-viral delivery strategies. The successful translation of these findings into clinical settings is now underway. In 2016, China initiated the first human trial using CRISPR-edited T cells to disrupt *PD-1* in patients with refractory lung cancer, reporting good tolerability and modest tumor control. A landmark Phase I trial in the United States by Stadtmauer et al. (2020) demonstrated the safety of multiplex CRISPR editing in T cells targeting *PD-1* and *TCR* genes in patients with advanced myeloma and sarcoma. More recent trials are exploring CRISPR as a tool for generating CAR-T cells with improved persistence and reduced exhaustion. While most current trials rely on ex vivo gene editing, early attempts at direct in vivo delivery of CRISPR plasmids into tumors have shown partial responses in liver and cervical cancer patients. Although clinical efficacy is still in the early stages, these studies collectively confirm that CRISPR-based cancer therapies are both feasible and safe, laying the foundation for more advanced and personalized treatment strategies in the near future.

III. ADVANTAGES AND CHALLENGES

Advantages	Challenges
High precision targeting	Off-target effects / unintended mutations
Personalized and versatile	Poor delivery to solid tumors
Long-term or one-shot cure potential	Immune reactions to Cas9 or vectors
Boosts immunotherapy efficacy	Tumor heterogeneity may limit success
Lower systemic toxicity	Ethical and regulatory hurdles

Future Perspectives of CRISPR in Cancer Treatment

The future of CRISPR-based cancer therapy is highly promising, with several emerging directions that could overcome current limitations:

1- Delivery Systems

One of the biggest challenges today is delivering CRISPR components safely and specifically to tumor cells. Future research is focusing on nanoparticle-based delivery, exosome carriers, and virus-free delivery methods to increase precision while minimizing off-target effects.

2-Personalized Cancer Medicine

With advancements in genomic sequencing, CRISPR could be customized for individual patients by directly correcting patient-specific mutations or engineering immune cells tailored to their tumor profile. This will shift treatment from generalized protocols to truly personalized therapy.

3-Next-Generation CRISPR Systems

Newer variants like CRISPR-Cas12, Cas13, Cas14, and base editors/prime editors offer higher accuracy with fewer cuts in DNA. These tools may enable gene correction without double-strand breaks, reducing risks like chromosomal instability.

4- Combination with Immunotherapy

CRISPR-edited CAR-T and NK cells are already in clinical trials. Future strategies may involve multi-gene edited immune cells with enhanced tumor recognition, improved persistence, and resistance to tumor immune suppression.

5-Targeting Tumor Microenvironment & Metastasis

Instead of only editing cancer cells, future approaches may use CRISPR to reprogram surrounding stromal cells or inhibit metastatic pathways, preventing recurrence and spreading.

6-Ethical AI-Driven CRISPR Design

Integration of Artificial Intelligence could allow prediction of the best editing sites and off-target risks before therapy is applied. This will enhance safety, regulation, and treatment planning.

7-In Vivo Gene Editing for Solid Tumors

Currently, most CRISPR therapies are ex vivo (done outside the body). The long-term goal is to inject CRISPR directly into tumors, allowing minimally invasive treatment of hard-to-access cancers like brain or pancreatic tumors.

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