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Applications and Challenges of CRISPR/Cas9 Technology in Cancer Treatment

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Abstract. The CRISPR/Cas9 system has revolutionized gene editing by offering a precise, efficient, and cost-effective method for targeting, modifying, and regulating genomic loci across diverse organisms. Initially discovered in bacteria, CRISPR/Cas9 has evolved into a powerful tool for cancer treatment, enabling both in vivo and ex vivo gene editing strategies. The system's ability to induce targeted DNA breaks and harness cellular repair mechanisms has facilitated significant advancements in genetic research and therapeutic applications. In cancer treatment, CRISPR/Cas9 shows promise in disrupting tumor survival genes and enhancing immune cell therapies. In vivo applications have demonstrated significant tumor inhibition and increased survival rates in preclinical studies, while ex vivo approaches, such as the modification of T cells for enhanced antitumor activity, have shown promising results in clinical trials. Despite its potential, CRISPR/Cas9 faces several technical and ethical challenges. Off-target effects, delivery system optimization, and ensuring the stability and safety of edited cells are critical technical hurdles. Future directions for CRISPR/Cas9 technology include developing new CRISPR systems with enhanced specificity, precise and efficient delivery methods, and multiplex gene editing capabilities. Integrating CRISPR with existing cancer treatments, such as immunotherapy and chemotherapy, can boost treatment efficacy and overcome drug resistance. In summary, CRISPR/Cas9 offers a promising future for cancer treatment through continuous development and refinement.

Keywords: CRISPR/Cas9, Cancer Therapy, Gene Editing, Immunotherapy, Technical and Ethical Challenges.

1. Introduction

1.1. Components and Functions of CRISPR/CAS9

The CRISPR-Cas system, first identified by Japanese researchers in 1987, was noted for its short direct repeats interspersed with genomic sequences in Escherichia coli. This groundbreaking microbial adaptive immune system has since been harnessed for precise genome editing [1]. The origin of the Type II CRISPR system is unique because it relies on the Cas9 nuclease, with which only CRISPR RNA (crRNA) and trans-activating crRNA will work. The crRNA contains the 20 nucleotide guide sequence which is complementary to the target DNA and thus guides Cas9 to a specific genomic locus by Watson-Crick base pairing [2]. In contrast, the tracrRNA helps to process crRNA into its mature form [2]. Central to the CRISPR/Cas9 system is the protospacer adjacent motif (PAM), a minimal region of homology located immediately downstream from the crRNA-targeted sequence in invading DNA. The specificity

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of Cas9 is maintained through the nature and identity between different PAM sequences among microbial species, which enables the crRNA-tracrRNA complex to bind its target efficiently [3]. Once the crRNA-tracrRNA complex recognize and bind to the target DNA sequence, Cas9 creates a double-stranded break (DSB) three base pairs upstream of the PAM site. This break triggers cellular repair mechanisms through non-homologous end joining (NHEJ) or homology-directed repair (HDR) [4]. The CRISPR/Cas9 system's ability to induce targeted DNA breaks and harness the cell's natural repair processes has made it an indispensable tool in genetic research, enabling scientists to study gene function, develop gene therapies with unprecedented accuracy.

1.2. Mechanism of Gene Editing Process

The design and execution of CRISPR systems necessitate meticulous methodologies to achieve effective gene editing. To increase the system's reprogram ability and simplicity, the crRNA and tracrRNA is combined into a single-guide RNA (sgRNA). Initially, a plasmid that contains both Cas9 gene and sgRNA scaffold is designed and transferred into the targeted cell. If HRR is desired, the guide sequence oligonucleotides are then cloned into the same plasmid [4]. This plasmid acts as a vector that delivers all necessary components.

After being delivered, the CRISPR/Cas9 components launch a three-stage adaptive immunity effort. In this process, invader DNA is introduced into the CRISPR array and transcribed as precursor CRISPR RNA (pre-crRNA), then pre-crRNAs are further cleaved to crRNAs and finally crRNAs guide Cas9 proteins for foreign DNA infection. When Cas9 binds to gRNA, its structure changes, allowing the RNA-DNA complex to travel through it. The C-terminal of Cas9 recognizes and binds its PAM on the target. It pairs with the target DNA strand, thereby releasing the non-target and separating the duplexed DNA into an R-loop [5, 6]. There are two different nuclease domains of Cas9: HNH and RuvC domains. The complementary DNA strand is cut by the HNH domain of Cas9, whereas a RuvC-like nuclease domain cleaves the non-complementary one; this results in double-strand break (DSB) formation [1].

DSBs induced by Cas9 activate cellular repair mechanisms that determines the gene-editing outcome. There are two primary pathways for DSB repair: Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). NHEJ is an error-prone pathway that processes and rejoins broken DNA ends without a homologous template, leading to random insertions or deletions (indels) at the break site. NHEJ results frameshift mutations and gene knockouts. Conversely, HDR offers a precise method for gene editing by using an external repair guiding template. HDR’s ability of designing repair templates allows introduction of specific mutations, gene insertions, or corrections, offering great flexibility and specificity [6].

1.3. Revolutionary Breakthrough in Gene Editing Technology

CRISPR-Cas9 offers a simple, efficient, and cheap method for programmable and precise gene editing across various organisms. Compared to earlier gene-editing technologies such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR-Cas9 provides notable advantages in terms of ease of use, efficiency, and flexibility. CRISPR-Cas9 makes precise blunt cut, requires only the modification of the guide RNA sequence to change target, and allows for multiplexing capabilities [8, 9]. In summary, the various advantages of CRISPR-Cas9 has transformed gene editing and presents vast potential for cancer treatment.

This study is designed to explore the novel applications of CRISPR/Cas9 technology in cancer treatment in more depth, considering both in vivo and ex vivo approaches to gene editing. Through understanding the approach’s mechanisms and results in CRISPR-mediated genome editing, unique opportunities to improve the accuracy and effectiveness of existing cancer treatment options will be identified. This work will draw on a combination of literature review and experimental data to address the potential and challenges of CRISPR/Cas9 technology when targeting cancer cells and modulating immune responses. The primary contribution of this research will be to discover how CRISPR/Cas9-based treatments can transform cancer therapy, offering treatment modalities that are both more effective

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and less toxic. By attempting to overcome the technical and moral obstacles, this work hopes to prepare for the successful achievement of CRISPR/Cas9 therapy implementation in the clinical setting.

2. Applications of CRISPR in Treating Cancer

2.1. In-Vivo Gene Editing Techniques

CRISPR/Cas9-mediated in vivo gene editing for cancer therapy holds great promise, but also presents remarkable opportunities and challenges. The most obvious advantage of in vivo gene editing is the theoretical capacity to permanently abrogate tumor survival genes. The traditional cancer treatment often required patients to receive multiple treatments, resulting in higher toxicity, treatment costs, and reduced quality of life. CRISPR/Cas9 gene editing, on the other hand, might do away with some or most of these treatment repetitions. It can interrupt the expression of a critical gene that provides tumors with their survival advantage by promoting apoptosis or inhibiting tumor growth resulting in increased treatment effectiveness and decrease in treatments needed. This strategy would then likely go a long way towards actual patient benefit and quality of life, without the side effects that are typically seen with standard chemotherapies or other drugs.

Although in vivo gene editing holds enormous promise, several important roadblocks must first be cleared. One of the major challenges for CRISPR/Cas9 technology is a highly efficient and tumor-selective delivery to their target cell. This is made difficult by the presence of strong selective pressures against edited cells in the tumor microenvironment, which results in poor editing efficiency(source). Moreover, CRISPR technologies concerning safety issues are yet to be guarded by broader risk off-target gene editing. These off-target consequences may cause bystander cells to exhibit unwanted mutations, thus increasing the risk of secondary cancers or other genetic diseases [10, 11]. Accordingly, exact and safe gene editing is central to the viability of CRISPR in clinical use.

CRISPR/Cas9 has been shown to be most effective at targeting particular cancers in an individual using engineered cancer xenografts. For example, Rosenblum et al. engineered a potent cationic lipid for programmed encapsulation of Cas9 mRNA and single guide RNAs (sgRNAs) in the same multiplex position by LNP delivery. They tested this system in two metastatic cell lines: 005 (murine glioblastoma) and OV8 (human ovarian carcinoma). In this context, PLK1 (Polo-like kinase 1), a conserved cell cycle regulatory serine-threonine protein kinase, is an important target since its disruption results in G2-M phase cell-cycle arrest and apoptosis mainly caused by defect in cytokinesis of dividing cells. Following a 1st generation (gen) screen, stk5936 morphants lacking full length PLK-1 developed embryos electively exhibiting increased apoptosis at the lens placode and neural tube by in situ hybridization to apoptotic marker gene. When combined systemically with irinotecan, this approach induced substantial cancer cell apoptosis and retarded tumor growth by 50% in addition to bettering survival rates by approximately 30 percent (Rosenblum et al., 2020).

To expand the utility of this technology to disseminated tumors, cLNPs with antibody targeting were developed. For instance, the i.p. injections of EGFR-targeted sgPLK1 cLNPs led to ovarian disseminated tumor-specific uptake. Up to 80% gene editing in vivo, significant tumor growth inhibition, and a survival rate increase of up to 80 was observed [12]. These data highlight the general utility of CRISPR/Cas9 for cancer therapy, and suggest that targeted gene disruption can be achieved in metastatic deposits using this approach. These results demonstrate the potential translation of CRISPR/Cas9 to in vivo therapeutics and identify avenues for improvement, also showing that this technology could become a powerful adjunct in cancer treatment.

2.2. Ex Vivo Gene Editing Approach

Ex vivo gene editing is the modification of cells outside of a body for in-body re-administration. In cancer treatment, this typically begins with T cell isolation from blood collected prior to immune system stimulation. These T cells are then treated with CRISPR-Cas9 ribonuclear protein complexes containing distinct sgRNAs. These complexes are delivered to T cells via electroporation which allows for highly targeted gene editing. A major feature of this approach is knockout immunosuppressive factors that are

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mainly programmed cell death protein 1(PD1) ligand in primary T cells. This deletion focuses on ramping up T cell activity by eliminating inhibitory signals and is currently under scrutiny for the same in adoptive therapies comprising of experimental models using Tumor-Infiltrating Lymphocytes (TILs) or Chimeric Antigen Receptor (CAR) T cells. The endogenous T cell receptor (TCR) is another important target of gene editing to avoid immune rejection and prevent TCR priming in allogeneic (donor-derived) T cells. This alteration prevents the rejection of T cells donated from a likewise named donor. Moreover, replacing a tumor-specific TCR or CAR element for the endogenous TCR may increase the specificity and efficacy of T cells in killing tumors [13]. Engineered T cells are validated by NGS post these modifications to show that there is a high on-target editing with little off-target specification. Following validation, these T cells are expanded in culture and ready for reinfusion into the patient. A crucial aspect during the testing of engineered T cells is that both their safety and efficacy are assessed by following the patient's disease course.

Stadtmauer et al. showed the power of this tool in tumor treatment with their research on fresh donated T cell ex vivo gene editing cases. Blood of cancer patients was utilized to isolate T cells and for CRISPR-Cas9-based gene editing in the present study. Different ribonuclear protein complexes were designed to target and edit the TRAC, TRBC1/TRBC2 loci or PDCD1 (encoding PD-1). To this end, they transduced the cells with a lentiviral vector expressing a T-cell receptor (TCR) that recognizes an HLA-A*0201-restricted NY-ESO-1157-165 peptide or LAGE0162. Those modified T cells were then reinjected into the patients intravenously. These T cells had been engineered to harbor deletions of the endogenous TCR and PD-1 to enhance antitumor immunity. Deletion of PD-1 also intended to be an immunosuppressive caution for autoimmune and associated toxicities. Expression of the NY-ESO-1 transgene then allowed recognition and specific targeting by the engineered T cells for tumors expressing those antigens. In this study, the T cells were well-tolerated in patients and yielded durable engraftment. A few off-target edits were observed in the study with > 30% of cells showing no mutations, original mutation clones (approximately 40%), a single mutated clone, and double- or triple-mutated colonies representing approximately 20% and about <10%, respectively, at target sequences. As such, the strategy of ex vivo gene editing described here may provide a roadmap for potent and highly specific cancer therapies with minimum off-target effects, which could lead to broader utility in treating diverse types of cancers [14].

3. Clinical Trails

3.1. Current Clinical Trial Summary

Clinically, CRISPR/Cas9 technology is actively being pursued for the treatment of different types of cancers, with most studies focusing on engineering immune cells as therapeutics. In cancer therapy, CRISPR/Cas9 can be divided into two general categories: in vivo gene disruption and ex vivo engineering of immune cells for immunotherapy.

There are relatively few existing clinical trials that have examined the possibility of direct in vivo knockout with CRISPR/Cas9. One interesting study, NCT03057912, targets the disruption of HPV16 and HPV18 E6/E7 DNA [15]. This perturbation has demonstrated substantial efficacy as it reduces the protein expression of oncogenic E6 and E7, causing apoptosis induction and thereby anti-cell proliferation in HPV-related tumors. This study is a major advancement in using CRISPR/Cas9 for genome editing in cancer cells and provides proof of principle that it can disarm oncogenic driver mutations at the DNA level.

Most CRISPR/Cas9-orchestrated clinical trials, on the other hand, focus on engineering immune cells to boost the body's cancer-killing response. These efforts are now emerging to use gene editing directly into patients. Most of these trials are in Phase I, illustrating their early stage although tentatively advancing field. Many trials have focused on antagonizing PD1 and releasing the natural brakes it exerts over immune responses, to enable anti-cancer host cells such as T Cells back into cancer repressive mechanisms. Notable trials in this area include NCT03081715, NCT02793856, NCT03044743, and NCT04417764[16-19]. Since PD1 limits an immune reaction, blocking it by these inhibitors frees this

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limitation to enhance the antitumor response. Building upon this, trial NCT03545815 takes it a step further and uses PD1 knockout along with TCR (T-cell receptor) knockouts [20]. The goal of this approach, which simultaneously eliminates two genes to help make the CAR-T cells more specific and effective against cancer cells, is to minimize off-target effects resulting from therapeutic cell killing while augmenting tailored immune-cell function.

Another paradigm involves CAR insertion with knockout of TCRs. One method being pioneered by clinical trials such as NCT04502446, NCT04244656, and more [21, 22]. These trials involve the addition of CARs to T cells and a simultaneous knockout of the endogenous TCR, arming T-cells with synthetic receptors that have an enhanced affinity for cancerous cells and are designed to prevent off-target immune responses. This tactic represents how CRISPR/Cas9 can tailor a number of genetic targets for exceptionally unique and potent cancer treatments. Collectively, the current CRISPR/Cas9 clinical trials in cancer treatment have emerged as a composition of direct gene editing and refined immunotherapy construction. The lessons learned from such trials are expected to enable new exciting treatment strategies that should greatly increase cancer therapy specificity and effectiveness, allowing more selective and less toxic therapeutic alternatives in the future.

3.2. Clinical Trial Data of a Preliminary Nature

Preliminary results of CRISPR/Cas9 clinical studies to cure cancer uniquely inform its safety and efficacy. The landmark study by Stadtmauer et al. used CRISPR-Cas9 to target and edit the TRAC, TRBC1, TRBC2, and PDCD1 loci in T cells expressing a NY-ESO-1/HLA-A*02:01-specific TCR. The on-target editing efficiency was quite impressive, with means of 99.4% for PDCD1, 98.6% for TRAC, and 95.8% for TRBC. Off-target mutations were still detected, but they seemed to have minimal clinical relevance and mostly affected genes like CLIC2, ZNF609, and LINC00377 without detectable biological consequences. No patients experienced clinical toxicities related to the engineered T cells. Chromosomal translocations detected in vitro at the time of cell manufacturing decreased post-infusion. All three patients exhibited T cell trafficking to tumor sites on biopsy. There were still tumor remnants, but a significant decrease in expression appeared for the target antigens NY-ESO-1 and LAGE-1 in myeloma patients, reflecting a partial effect [14].

The study's major victory was proving T-cell trafficking to the tumor and diminished target antigens, indicating antitumor action. The lack of autoimmunity or T cell genotoxicity after the engraftment of PD-1 deficient T cells is encouraging, as there have been concerns about autoimmune reactions and genetic damage from inhibiting this signaling molecule. However, they also identified several obstacles. Although not statistically significant at the present, concerns regarding off-target mutations highlight the importance of proper control over gene editing. The continued risk of chromosomal translocations during cell manufacturing that decreases with time should be assessed in more detail. A second problem is previous immune responses that could lead to rejection of the engineered cells (not detected in this study but a concern for next steps). The prevalence of 30%-digenic and trigenic-edited cells in the infused population (20% persistence after four months) suggests editing efficiency as well as cell longevity will need further optimization.

In summary, multi-pronged genome editing by CRISPR/Cas9 appears to be a clinically translatable concept based on early clinical results. The early safety profile seems to be good, but more experience and longer follow-up in larger series of patients will need to demonstrate the full potential and limitations. Much is riding on the successes and failures found in these early attempts to refine CRISPR/Cas9-based therapies for potent but safe cancer applications.

4. Technical and Ethical Challenges of CRISPR in Cancer Treatment

4.1. Technical Challenges

CRISPR/Cas9 for cancer therapy shows great potential but also faces a few technical hurdles, the biggest being undesirable off-target effects. These off-target genetic changes can result in unintended, possibly dangerous or unhealthy effects. To ensure maximum specificity there needs to be presence of PAM, as

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well as homology between a single-guide RNA (sgRNA) and target DNA. Cas9 can also be inactivated when the target site resides within closed chromatin regions or its surrounding CpG dinucleotides are heavily methylated [23].

The stability and safety of CRISPR/Cas9 components or cells that are engineered by the technology is a prerequisite for therapeutic application. Furthermore, being immunogenic, the nucleic acids and proteins of gRNA and Cas may also provoke immune responses in vivo, affecting gene editing efficiency [13]. Existing Cas9 immune responses from in vivo editing could potentially preclude the use of CRISPR-engineered immune cells if infused back to the patient following ex-vivo modifications. One potential approach is to administer immunosuppressants to dampen these immune responses. Additionally, DNA repair pathways and structural variations (e.g., chromosomal translocations or large chromatin deletions) pose challenges to editing efficiency as well as genome stability [13]. They also have practical limitations such as the inefficiency of homologous direct repair (HDR) to fix double-strand breaks and the partial inability to deliver larger DNA constructs. Monitoring and suppressing chromosomal translocations are important to guarantee safety, as is profiling off-target changes in the host genome.

Cas9/sgRNA delivery optimization is another critical issue. Different delivery systems are being studied, including the use of viruses, plasmids, mRNA, and nanoparticles. It nevertheless continues to represent a major challenge in developing an appropriate system for gene delivery and ultimately proving them as safe systems with high efficiency while targeting the right tissues and cells. Novel delivery systems have been designed for in vitro as well as in vivo applications, but accomplishing all of the desired efficiencies at once has proven to be challenging. More improvements in delivery technologies are needed to expand the clinical utility of CRISPR/Cas9 in cancer therapy.

4.2. Ethical Challenges

The use of CRISPR/Cas9 for medical treatment, particularly in cancer therapy, raises serious ethical questions that need to be considered. Safety is a major concern, particularly the risk of off-target editing, where some cells are edited incorrectly. This carries a risk of accidental and potentially harmful genetic edits. Once genome editing technologies become a reality, issues of justice and equity will arise, as access to such technology may be limited to those who can afford it, creating social distinctions between genetic “classes” depending on the extent of genetic modifications. Moreover, moral and religious concerns about gene editing further complicate the ethical landscape. Additionally, the slippery slope argument posits that using gene editing for therapeutic purposes could eventually lead to non-therapeutic modifications (e.g., "designer babies"), raising fears of a new form of eugenics and marginalizing those without access to these technologies. Ensuring full information disclosure and safeguarding privacy in CRISPR/Cas9 research are indispensable for patients. The FDA regulatory framework outlines meticulous instructions for obtaining consent, minimizing confusion and misunderstandings.

Although CRISPR/Cas9 shows great promise for developing more effective cancer treatments with fewer side effects, ethical challenges must be addressed for its responsible and equitable implementation. Ensuring safety, justice, informed consent and privacy protection are essential for the ethical application of this groundbreaking technology.

5. Long-term Strategies and Outlook

Overall, CRISPR/Cas9 offers significant promise for cancer treatments. Genome editing with CRISPR/Cas9 is faster, more affordable, and superior to traditional methods. CRISPR technology could revolutionize complicated cancer therapies through its capacity to aim many genes at the same time, hitting the genetic heterogeneity and multifacetedness of malignancies squarely. This strategy may help shrink tumors and surmount medication opposition. In any case, it additionally presents wellbeing concerns and test difficulties as it moves into human trials. Continuous examination is expected to enhance techniques for influencing a few genes so as to amplify helpful outcomes while ensuring patient security.

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CRISPR-based editing holds promise for personalizing cancer care when partnered with standard treatments. Preliminary research shows how CRISPR can address genetic factors fueling cancer's growth, priming the immune system to better attack tumors. Such synergies may reverse disease progression and thwart resistance in ways single therapies cannot. Autologous treatments, where a patient's own cells are genetically modified to correct mutations, can avoid the rejection issues associated with donor transplants. This approach is particularly promising for disorders that can be addressed by modifying accessible cells from the patient, offering a highly personalized treatment option.

One key direction is strengthening the repertoire of CRISPR functionalities beyond basic gene knockout. CRISPR can also assist studies in revealing how singular genes function in cancer cell behavior and supporting new-generation immunotherapy. It facilitates the research of recurrent encoded variants and the identification of noncoding and regulatory elements in tumorigenesis. Such advances continue to push forward understanding and treatment of diseases. Moreover, exploring the use of CRISPR to control gene expression for genetic modulation and its effects on the tumor microenvironment (TME) as well as the immune system can offer a more systematic strategy to refine the approach in developing CRISPR-controlled cancer therapies.

In summary, the future of CRISPR technology in cancer treatment envisions constant evolution and optimization. Envisioning CRISPR amalgamated with budding imaging and sensory innovations, cultivating collegial collaborations between education and enterprise, and experimenting with complementary treatments will propel progress of CRISPR-centered cancer interventions. Such ambitions provide novel anticipation for efficacious, personalized, and precise malignancy remedies, in the long run bettering affected individual consequences and offering a more hopeful prospective for malignancy direction.

6. Conclusion

CRISPR/Cas9 technology has made significant advances in cancer treatment, offering a powerful tool for precise genome editing. The ability to target specific genes with high accuracy has opened new avenues for developing cancer therapies, particularly through the modification of immune cells and the direct disruption of tumor survival genes. This technology has shown promise in both in vivo and ex vivo applications, demonstrating significant tumor inhibition and enhanced antitumor activity in preclinical and clinical studies. Despite its potential, CRISPR/Cas9 faces several technological challenges, including off-target effects, delivery system optimization, and ensuring the stability and safety of the edited cells. Ethically, the technology raises concerns about equitable access, potential misuse for non-therapeutic enhancements, and moral objections to gene editing, particularly involving human embryos. Additionally, the long-term effects and potential unintended consequences of gene editing remain areas that require thorough investigation and continuous monitoring.

To advance CRISPR/Cas9 technology, it is crucial to foster innovation and promote interdisciplinary collaboration. Research should focus on developing new CRISPR systems with enhanced specificity, creating more efficient delivery methods, and exploring the integration of CRISPR with other therapeutic approaches. Collaborative efforts between academia, industry, and regulatory bodies will be essential in accelerating the translation of CRISPR-based therapies from the lab to the clinic. Long-term studies are essential to fully understand the safety and efficacy of CRISPR/Cas9-based therapies. Research should not only focus on immediate outcomes but also on the potential long-term impacts of gene editing on patients' health.

In summary, the future of CRISPR technology in cancer treatment involves continuous development and refinement. Integrating CRISPR with emerging imaging and sensing technologies, fostering collaborative efforts between academia and industry, and exploring combination therapies will drive the advancement of CRISPR-based cancer treatments. These efforts will provide new hope for effective, personalized, and precise cancer therapies, ultimately improving patient outcomes. By addressing both the technological and ethical challenges, CRISPR/Cas9 can become a cornerstone of modern cancer therapy, offering innovative solutions to some of the most pressing challenges in oncology.

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