The use of CRISPR/Cas9 in cancer immunotherapy

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Abstract

Cancer immunotherapy, particularly CAR-T cells, has transformed the landscape of cancer treatment, offering a paradigm shift in prognosis. To enhance the efficacy of cancer treatment, recent scientific endeavors have concentrated on harnessing the potential of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) technology where they use this technology to cancer treatments and cancer immunotherapy including the engineered CAR-T cell. There have been a lot of advances including correcting genetic mutation, curing genetic disorders, etc. this review paper will cover cancer immunotherapy, CAR-T cell therapy and it;s mechanism, successful clinical trials, ongoing clinical trials, the current limitations as well as the future advantages of this technology.

Introduction

In 1987, a strange DNA pattern was discovered in E. Coli. This pattern of clustered, regularly interspaced short palindromic repeats (CRISPR), would serve as the foundation for a new era in science and medicine - gene editing. A key component of CRISPR-based gene editing is CRISPR associated protein 9 (Cas9), which recognizes and cuts specific pieces of DNA. (S) Cas9 is programmed to target sites by the single guide RNA (sgRNA) and results in a double-stranded DNA break (DSB). Unlike other gene-editing technologies, such as zinc finger nucleus (ZFN) and transcription activator-like effector nucleus (TALENS), CRISPRcan target and cleave specific genes.

CRISPR/Cas9 has a wide variety of applications. It has been used to uncover novel cancer mechanisms and treatments, including gene manipulation, tumor immunotherapy, and drug resistance. Scientists discovered that CRISPR has a special role in bacteria defense systems like bacteriophages, kills and selectively targets bacteria, and is also a drug resistance bacteria, and plasmid conjugations. The bacteria can incorporate the spacer sequence to get a better immune system and adapt to the phage resistance. The protospacer within the virus DNA is homologous to the spacer sequence in the bacteria's DNA that comes from the phage gene of CRISPR. This is identified as PAM (palindrome adjacent motif) and will be explained in detail in the next section.

In this paper, we will be taking a look at the CRISPR/Cas9 technology in detail, with a focus on its application in cancer immunotherapy. We will examine a few case studies from each and take a look at the implications of each immunotherapy approach.

Understanding CRISPR Technology

CRISPR was first discovered in 1987 when a strange pattern in the gene of E. coli was noticed by research scientist Yoshizumi Ishino. This pattern contained 30 base pairs (bp) of palindromes, sequences of DNA that read the same both backward and forward. This sequence is commonly found in many microbes, including both domains of prokaryotes. Therefore, the term ‘CRISPR’- clustered regularly interspaced short palindromic repeats- was introduced. The CRISPR complex comprises 3 components: the CRISPR-associated protein 9 (Cas9), CRISPR RNA (crRNA), and tracer RNA (tracRNA). Each plays a key role in CRISPR-Cas immunity, which has three stages: adaptation, expression/maturation, and interference.

Upon viral infection, Cas1 and Cas2 enzymes engage in the cleavage of the protospacer region within the viral DNA. Subsequently, the excised protospacer fragment becomes affixed to a specific locus within the bacterial chromosome recognized as the CRISPR array. Notably, the protospacer integration transpires at the 5' terminus of the complementary end of the CRISPR array, concomitantly followed by the conformation of a novel repeat region. Cas 1 and Cas 2 enzymes effectuate the cleavage of the spacer at the site precisely termed the protospacer adjacent motif (PAM). Detection and identification of this motif are facilitated by the nucleotide sequence NGG, wherein the "N" designates any nucleotide, followed by two consecutive guanine residues. This specific motif pattern is exclusive to viral DNA and does not manifest within bacterial genomes. Through this discriminative PAM recognition mechanism, the catalytic activities of Cas1 and Cas2 are engaged, thereby ensuring precision in targeting the protospacer while precluding unintended cleavage of bacterial DNA. This phenomenon is formally acknowledged as the process of adaptation[1].

Furthermore, during the expression/maturation phase, the RNA polymerase transcribes the entire CRISPR array, generating a precursor RNA molecule termed pre-crRNA. This pre-crRNA encompasses a sequential arrangement of repeats and spacers, along with unprocessed synthetic tracRNA. The tracRNA is synthetically constructed and comprises segments that exhibit complementarity with the CRISPR RNA sequence[2]. After this interaction, facilitated by base pairing, the tracRNA aligns with the repeat region of the pre-crRNA. After this alignment, enzymatic activity attributed to RNAase mediates the cleavage of both the repeat region and the spacer, conjoined with the associated tracRNA segment, ultimately culminating in the formation of mature crRNA. Notably, the artificially generated tracRNA serves an analogous role to RNAase, functioning to enzymatically divide the pre-crRNA into discrete crRNA fragments[2].

Subsequently, the Cas9 protein, constituting a solitary polypeptide chain, manifests six principal domains denoted as Recognition 1 (REC1), Recognition 2 (REC2), Bridge Helix (BH), PAM-Interacting (PI), HNH, and Ruvc. Notably, the PI domain assumes the critical role of discerning the Protospacer Adjacent Motif (PAM), while the HNH and Ruvc domains function as the nuclease moieties responsible for effectuating DNA cleavage within the Cas9 protein context. In laboratory settings, an alternative strategy involves using single-guide RNA (sgRNA) instead of tracRNA, as previously mentioned. The Cas9 molecule subsequently engages with the associated crRNA upon encountering viral DNA. Upon their fusion, the fusion structure transitions to being designated as the guide RNA (gRNA). Facilitated by REC1 interactions, the Cas9 enzyme undergoes activation upon binding with sgRNA. Subsequently, an exploration phase ensues, during which the Cas9-gRNA complex employs the PI domain to search for a complementary DNA sequence at the targeted site, which corresponds to the PAM site. Upon successful identification, the Cas9-gRNA complex initiates DNA unwinding, assessing the degree of complementarity between the gRNA and the sequence proximal to the PAM on the opposing DNA strand. Upon establishing complementary correspondence, the Cas9-gRNA complex invokes the Ruvc and HNH domains, resulting in a dual-strand cleavage event, effectively inducing a double-strand break (DSB) within the target DNA locus[3].

CRISPR/-Cas9's exquisite accuracy in homing to pinpointing specific DNA sequences empowers scientists to devise gRNAs to guide RNAs that escort the Cas9 enzyme to predetermined sites within the genome. Upon arrival, Cas9 initiates the formation of a double-strand break in the DNA. Subsequent reparative processes can introduce deletions or insertions, causing disruptions in the gene's reading frame and yielding a dysfunctional protein product.

Following the induction of double-strand breaks (DSBs), the genome repair process unfolds via two distinct pathways: Non-homologous End Joining (NHEJ) and Homology Directed Repair (HDR). In the context of NHEJ, an integral protein entity known as Ku70/80 exhibits pronounced affinity for the terminal extremity of DNA fragments. Cooperative interaction with DNA Protein Kinase Catalytic Subunit (DNA-PKcs) ensues, resulting in a complex formation at the Ku70/80 locus. In conjunction with a constellation of spring-like proteins enveloping the terminal DNA ends, this intricate assembly orchestrates their fusion. After this alignment, the enzyme DNA Ligase 4 takes center stage, catalyzing the formation of phosphodiester bonds between the juxtaposed DNA ends. Notably, the NHEJ mechanism obviates the requirement for an external DNA counterpart to serve as a template, as DNA Ligase 4 effectively bridges the fractured DNA termini in a direct linear continuum[4].

This biochemical orchestration, known for its inherent ability to expedite genetic alterations, is harnessed by researchers to elicit targeted gene mutations, thereby inducing gene deactivation of DNA structure.

Moreover, within the framework of Homology Directed Repair (HDR), in contrast to the NHEJ mechanism, a reliance on homologous DNA sequences becomes necessary. The gene editing process underlying HDR encompasses two discernible procedural pathways. In the first process, denoted as Synthesis Dependent Strand Annealing (SDSA), a discontinuity arises in the DNA strand, simultaneous with the availability of a homologous counterpart. This phase is paralleled by the Resection to Chi (RecBCD) process, wherein the 5' terminus of the disrupted DNA strand undergoes controlled degradation catalyzed by the RecBCD enzyme. This degradation ensues in a "T" shape excision, persisting until encountering the chi site – a distinct DNA stretch characterized by the sequence CGTGGTGGA. Subsequently, the Rec A enzyme intervenes, seizing hold of the 3' segment and directing its translocation toward the homologous DNA sequence, eventually locating the analogous region. DNA polymerase activity is then engaged to extend the 3' terminal end until its alignment with the chi site is achieved, resulting in the reconstitution of the original DNA strand[4].

The second process, recognized as Double-Strand Break Repair (DSBR), the simultaneous involvement of both 3' and 5' terminal ends is manifest. As the d loop structure is established, DNA polymerase catalyzes the extension of both termini, mirroring the previously mentioned mechanism. A cleavage event ultimately transpires, enabling genetic material insertion between the resultant DNA fragments. This coordinated interplay suggests chromosomal cross-linking, an elaborate expression suggestive of complex chromosomal interactions.

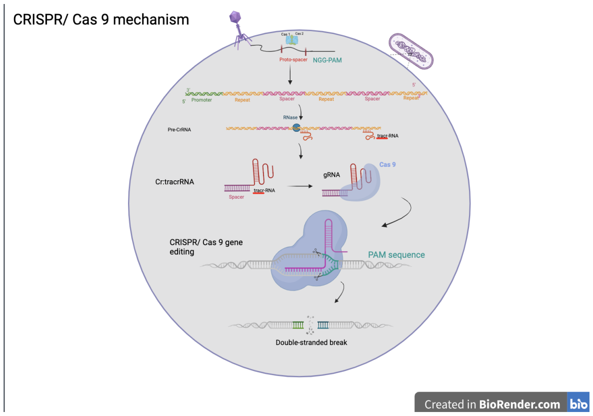

Fig. 2 How CRISPR works. This figure reviews the simple structure of CRISPR/Cas9 and its mechanism in gene editing-DNA.

How CRISPR can be used to modify genes and target cancer cells

CRISPR technology is being harnessed for a variety of cancer applications. A prominent application involves gene knockout and inactivation, whereby deliberate introduction of mutations yields loss of gene function. Oncogenes, which are gain-of-function mutations in cancer cells that induce or sustain malignancy, are often the target of CRISPR loss of function applications. By using CRISPR/Cas9 to inactivate or silence oncogenes,, researchers curb the unchecked proliferation and dissemination of cancer cells. This strategy offers a focused approach to counteracting cancer at its core genetic level.

The management of CRISPR-based gene modulation relies upon the fusion of the Cas9 protein with functional domains that are inherently conducive to either transcriptional activation or inhibition. Termed activators and repressors, these domains can be thoroughly tailored to engage with the specific regulatory loci of the target gene, thus introducing deliberate modifications to its expression profile.

Gene correction and repair are another application of CRISPR in the context of cancer. Inherited genetic disorders and syndromes predisposing individuals to cancer often originate from specific mutations within pivotal genes. The proficiency of CRISPR-Cas9 in correcting these mutations holds promise in addressing the fundamental genetic etiology of these diseases. Individuals hosting distinct genetic irregularities are vulnerable to particular cancer types. A notable example are mutations in the BRCA1 and BRCA2 genes, which markedly heighten the susceptibility to breast and ovarian cancers. By leveraging the precision of CRISPR-Cas9, researchers possess the capacity to carefully introduce accurately rectified DNA sequences into patient-derived cells, potentially enhancing the tendency for cancer emergence[5]. The gene correction process encompasses delivering a template DNA sequence coexisting with the CRISPR-Cas9 constituents. During repair, the cell may utilize the provided template to faithfully substitute the mutated sequence, thereby reinstating the normative gene function to counteract aberrant cellular processes.

CRISPR-Cas9's utility also extends beyond gene knockout and repair; it may also be employed to tune gene expression. This involves targeted modification of the regulatory components governing gene activity, allowing for the designated gene's heightened (activation) or attenuated (inhibition) expression. The management of CRISPR-based gene modulation relies upon the fusion of the Cas9 protein with functional domains that are inherently conducive to either transcriptional activation or inhibition. Termed activators and repressors, these domains can be thoroughly tailored to engage with the specific regulatory loci of the target gene, thus introducing deliberate modifications to its expression profile. The distribution of gene expression equilibrium is a cardinal feature in the pathogenesis of various diseases, including cancer. By employing CRISPR-Cas9 to modulate gene expression gradients, researchers are poised to influence the behavioral attributes of cancer cells. For example, the repression of genes pivotal in instigating cellular proliferation or the activation of genes directly programmed cell death may emerge as strategic therapeutic avenues for limiting the trajectory of cancer progression[6].

CRISPR Applications in Cancer Immunotherapy

Following the elucidation of CRISPR/Cas9's foundational mechanisms in the preceding section, now the focus is on one of the most pivotal applications of this groundbreaking technology: cancer immunotherapy. The precision and versatility of CRISPR/Cas9 have ignited a paradigm shift in the field of oncology, enabling the development of tailored therapies that harness the immune system's intrinsic capabilities to target and eradicate cancer cells.

Another utilization of CRISPR/Cas9 is to enhance immune responsiveness. T cells stand as pivotal constituents of the immune framework, tasked with recognizing and eliminating unhealthy, mutant cells, such as cancer. The therapeutic paradigm of CAR T-cell therapy exploits the inherent capacity of T cells to identify specific antigens presented on the surface of cancer cells. This approach involves genetically modifying T cells to express chimeric antigen receptors (CARs), thereby furnishing these immune effectors with heightened precision in target recognition. The composition of CARs integrates an antigen-binding domain derived from an antibody with intracellular signaling modules. This engineering blueprint instills CAR T cells with the capability to detect cancer-associated antigens, provoking a strong immune reaction that encompasses T-cell activation, expansion, and cytotoxicity directed toward the designated cancer cells. The integration of CRISPR-Cas9 technology engenders a refinement of precision and efficacy within the scope of CAR T-cell therapy, achieved by permitting researchers to tailor the genetic attributes of CAR T cells. The inherent versatility of CRISPR-Cas9 empowers scientists to thoroughly modulate diverse facets of T-cell dynamics, thereby optimizing therapeutic outcomes through informed customization[7].

Adoptive Cell Therapy (ACT) represents a promising approach in cancer treatment, involving the infusion of specialized lymphocytes derived from a patient's peripheral blood. There are three main categories of ACT: Tumor-Infiltrating Lymphocytes (TIL), T Cell Receptor (TCR) manipulation, and Chimeric Antigen Receptor (CAR)-engineered T cells. Lymphocytes, a type of white blood cell, play a crucial role in immune responses and differentiate into T and B cells. TILs are isolated from tumor biopsies and infused into patients to target cancer cells, often supplemented with immune-modulatory agents like interleukin-2. However, obtaining TILs from tumor tissue remains challenging (Ghaffari, Khalili, and Rezaei 2021, 3-5). TCR-based ACT relies on T cell receptors recognizing antigen-presenting molecules on target cells, with TCRαβ being the predominant subtype. The TCR activation process involves a complex protein arrangement known as the CD3 complex[8]. CAR-T cell therapy, on the other hand, utilizes engineered T cells to seek out cancer cells through Chimeric Antigen Receptors, which recognize specific cancer cell antigens. This approach involves the fusion of the CAR and T cell, creating CAR-T cells that concentrate at cancer sites and recruit other T cells, aided by cytokine signaling proteins, overcoming the evasion mechanisms of cancer cells[9].

Modulating immune response using CRISPR to enhance cancer immunotherapy

The concerted action of the CAR-T cell and the cytokines concludes in the termination of the cancer cell.The source of T cells for CAR-T cell production can be the patient's own cells (autologous) or cells from a donor (allogeneic). Blood is collected from the patient or the donor through venipuncture or apheresis. After purification, T cells undergo genetic manipulation, typically entailing the introduction of CARs through the transduction of patient T cells with viral vectors harboring the requisite DNA constructs[9]. Following genetic engineering, CAR T cells are expanded outside the body (ex vivo), employing non-viral techniques to eliminate the expression of proteins such as HLA class 1 and 2, particularly in allogeneic T cells. This strategic modulation serves to mitigate host immune rejection. However, inherent limitations exist: autologous CAR-T cell therapy encounters challenges in procuring sufficient T cell numbers from patients who are lymphopenic due to prior treatments, while allogeneic therapy poses the risk of the patient's immune system rejecting donor-derived cells, thereby potentially causing toxicity or triggering graft-versus-host disease (GvHD)[8].

Consequently, CRISPR technology offers a diverse array of avenues to enhance the safety and efficacy of CAR-T cells. It enables precise knock-in of the Chimeric Antigen Receptor (CAR), facilitating targeted integration. Furthermore, CRISPR can execute gene knockout strategies within the CAR-T cells to augment their cancer cytotoxicity. Additionally, CRISPR methodologies can be leveraged to effect edits that streamline CAR-T cell attributes, optimizing both scale and extended proliferation potential. Finally, CRISPR-mediated techniques can generate "universal" CAR-T cells derived from induced pluripotent stem cells, thereby presenting a strategy to alleviate the limitations of antigen specificity.

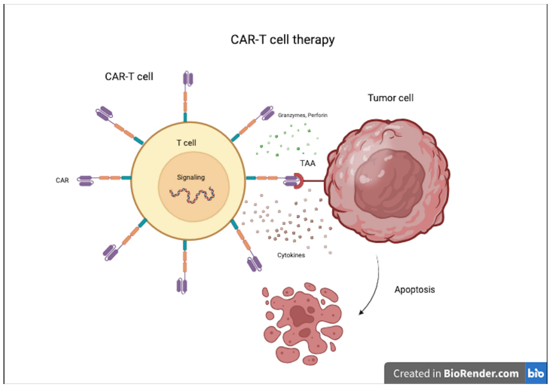

Fig. 3 CAR-T cell therapy. This figure shows the engineered T cells with attached CARs on the surface of the T cell. Through this process the CAR-T cell is bonded with the tumor cell releasing cytokines and perforin that eventually leads to the apoptosis of the tumor cell.

The application/effect of CRISPR-based immunotherapy for cancer treatment

In comparison to alternative gene editing methodologies such as ZFN and TALENs, CRISPR stands out for its enhanced simplicity, precision, and operational efficiency. Within the domain of cancer therapy, CRISPR offers the potential to augment the anti-tumor efficacy of CAR-T cells by optimizing their production process and enabling the creation of allogeneic CAR-T cells devoid of TCR beta chain-associated graft-versus-host disease (GVHD).In the context of cancer immunotherapy, CRISPR/Cas9 screening has been employed to identify pivotal genes implicated in the functionality of T cells[10]. This approach involves a two-cell assay employing human T cells as effectors and melanoma cells as targets. Utilizing a genome-scale CRISPR-Cas9 library encompassing 123,000 sgRNAs, researchers profiled genes whose disruption in tumor cells compromised the effector functions of CD8+ T cells. They contributed to resistance against T cell-based immunotherapy. Consequently, hitherto unexplored genes and microRNAs were discovered to promote T cell-mediated tumor damage. Furthermore, CRISPR screening was instrumental in unraveling the mechanism of T cell activation, ultimately identifying FAM41B as a novel target for tumor immune interventions[10].

The application of CRISPR/Cas9 within the domain of CAR-T cell therapy has exhibited notable promise across diverse tumor types, including hematological malignancies. Noteworthy advancements have been made in treating B-cell malignancies, exemplified by a remission rate of 90% in the case of CD19-specific CAR-T cells for acute lymphoblastic leukemia. Despite these successes, the production of universal "off the shelf" CAR-T cells from healthy donors faces challenges, including graft-versus-host disease (GVHD) and safety concerns related to potentiated immune activity. The recognition of recipient alloantigens by the allogeneic CAR-T cell's αβ T-cell receptor (TCR) can elicit GVHD due to HLA incompatibility[10]. CRISPR technology has also facilitated the generation of CAR-T cells with modified TCR beta chains, resulting in CAR-T cells that retain desired antitumor functionality while circumventing GVHD concerns. Consequently, CRISPR-directed methodologies offer a promising avenue for generating universal CAR-T cells[10]. Additionally, CRISPR has demonstrated its capability to enhance the anti-tumor effect of CAR-T cells, particularly through double and triple knockouts (e.g., TRAC, B2M, and PD-1), which exhibited heightened anti-tumor activity. By disrupting T cell inhibitory receptors such as PD-1 and LAG-3, CRISPR-modified CAR-T cells have showcased improved anti-tumor efficiency against hepatocellular carcinoma[10].

However, safety concerns surround CRISPR/Cas9 technology, primarily related to off-target mutagenesis and potential oncogenic activation. Off-target effects may lead to alterations in tumor-suppressor genes or the activation of oncogenes. Notably, p53-mediated DNA damage response has been observed in human retinal pigment epithelial cells following Cas9 RNA delivery, suggesting potential risks of chromosome rearrangements and oncogenic mutations.

Comparing CRISPR-based immunotherapy with traditional cancer treatment approaches

CRISPR-based immunotherapy strategies entail the targeted genomic modification of immune cells, primarily T cells, to augment the immune system's capacity to detect and combat cancer cells. CRISPR technology, distinguished by its exceptional precision in genome editing, allows for the specific manipulation of distinct DNA sequences. This precision substantially reduces the likelihood of unintended genetic alterations and off-target consequences. Consequently, CRISPR-based immunotherapy introduces a notably focused approach in contrast to conventional therapeutic modalities. Engineered immune cells modified with CRISPR exhibit heightened cytotoxicity and an elevated capability to identify cancer cells. Furthermore, CRISPR-engineered T cells can be equipped with chimeric antigen receptors (CAR-T cells), amplifying their aptitude for targeting cancer-specific antigens. This proactive immune response contributes to the destruction of tumor cells and fosters the establishment of immunological memory, potentially deterring relapse. Importantly, the adaptable nature of CRISPR technology allows for personalization, tailoring interventions to individualized cancer attributes, thereby presenting a promising avenue that surpasses conventional cancer treatments in efficacy[11].

However, challenges persist in the realm of CRISPR/Cas9 application. The efficient conveyance of CRISPR components into immune cells, including gRNA and Cas9 protein, into immune cells remains a formidable obstacle. Strategies to address this issue encompass viral vectors, electroporation, and nanoparticle delivery systems. Ensuring accurate genome editing while concurrently minimizing off-target effects is paramount to uphold safety standards[11].

Chemotherapy employs cytotoxic agents to disrupt essential cellular functions, impeding the division of cancer cells. Radiation therapy employs high-energy radiation to induce damage in the DNA of cancer cells, impairing their ability to undergo proliferation. Surgical interventions encompass the physical excision of tumors and affected tissue.

However, traditional therapeutic modalities lack the requisite specificity, exerting their effects indiscriminately upon both malignant and healthy cells. This indiscriminate impact leads to prevalent adverse effects, including nausea, anemia, and compromised immune function, thereby compromising the holistic well-being of patients. While conventional treatments can effectively diminish tumor size and eradicate cancerous cells, their prolonged administration might engender the emergence of drug-resistant phenotypes. Furthermore, these established approaches do not inherently bolster the patient's immune response vis-à-vis cancer cells. An exception is radiation therapy, which can potentially induce immunogenic cell death, thereby potentially provoking immune recognition. Moreover, these traditional modalities often fail to confer sustained immune memory responses, contributing to the vulnerability of relapse.

Clinical Case Studies: CRISPR in Cancer Treatment

In 2017, the Food and Drug Administration (FDA) approved the utilization of Yescarta and Kymerah. Subsequently, in 2018, both therapies received approval from the European Medicines Agency (EMA). Yescarta constitutes an FDA-endorsed immunotherapeutic modality engineered to potentiate the intrinsic antineoplastic capabilities of the individual's immune system[12]. Specifically categorized as a chimeric antigen receptor T-cell (CAR-T) therapy, Yescarta orchestrates the augmentation and proliferation of host T cells possessing the capacity to selectively eliminate malignant cells. Indicated for individuals who have large B-cell lymphoma, a discrete subset of non-Hodgkin lymphoma, Yescarta is intended for cases where the condition has either relapsed (reverted from remission) or proved refractory (resistant to remission) following a minimum of two conventional interventions, such as chemotherapy.The therapeutic mechanism entails an initial leukapheresis procedure to harvest a fraction of the patient's T cells, paralleling the blood donation. These acquired cells subsequently undergo ex vivo expansion, resulting in a vast population of potent antineoplastic T cells. Concurrently, the patient undergoes a three-day chemotherapy regimen to create a milieu conducive to the engraftment and efficacy of the newly fortified T cells. Ultimately, the re-engineered T cells are reintroduced intravascularly through intravenous infusion.Nonetheless, the therapy is accompanied by a spectrum of side effects, including cytokine release syndrome (CRS), arising from an exuberant immune response. This syndrome often manifests as flu-like symptoms (fever, headache, nausea), hemodynamic instability and respiratory distress. The manifestation of these side effects generally assumes a mild character. However,severe instances with potentially life-threatening consequences have been documented, particularly evidenced by a 13% incidence of significant CRS in the pivotal clinical trial (ZUMA-1) underpinning FDA approval. Additional neurologic sequelae might also arise[13].

The ZUMA-1 clinical trial enrolled 108 participants who were monitored for at least one year. Upon reaching the one-year milestone, 58% of these subjects achieved complete remission, with 24% demonstrating a partialtherapeutic response[13].

Kymriah, akin to Yescarta in its therapeutic modality, diverges in its application by encompassing not only adults who have large-B-cell lymphoma but also encompasses individuals under the age of 25 diagnosed with acute lymphoblastic lymphoma (ALL) marked by relapsed or refractory status. The procedural underpinning of Kymriah mirrors that of Yescarta. Correspondingly, the spectrum of side effects aligns, yet Kymriah introduces supplementary adverse events, including diarrhea, emesis, hypotension, vertigo, and cognitive perplexity[14].

The pivotal clinical trials substantiating FDA and EMA endorsements for ALL revealed that among a cohort of 63 participants, a noteworthy 83% (52 patients) achieved comprehensive remission—denoting absence of malignant evidence—within 3 months post commencement of treatment. In the context of large-B-cell lymphoma, the II-JULIET clinical trial instrumental in securing FDA authorization for Kymriah showcased outcomes from 106 participants, elucidating a complete remission rate of 32%, alongside an additional 18% attaining partial remission[14].

Subsequently, in 2020, the European Medicines Agency (EMA) approved Tecartus, a therapeutic intervention designed to address mantle cell lymphoma and large-B-cell lymphoma in adult patients, akin to Yescarta.However, a pivotal distinction delineating Tecartus from Kymriah and Yescarta resides in the underlying immunomodulatory mechanism, as the former employs a CD28-based construct, divergent from the 4-1BB-based architecture characterizing Kymriah. Furthermore, the manufacturing process of Tecartus diverges from that of its counterparts, Yescarta and Kymriah. In the therapeutic paradigm of Tecartus, the process entails the procurement of a subset of the patient's T cells through leukapheresis. These cells subsequently undergo laboratory-based refinement, encompassing the elimination of circulating tumor cells through white blood cell enrichment. A genetic modification is then introduced into the T cells, augmenting their capability to target and eliminate cancerous cells. After genetic manipulation, these cells undergo exponential replication, culminating in a vast cohort of T-cell warriors poised to combat malignancies. Subsequent stages of the treatment protocol closely parallel those of Yescarta and Kymriah. Equally, the constellation of side effects manifesting from Tecartus administration mirrors those observed with Yescarta[15].

The regulatory endorsement of Tecartus materialized in response to compelling evidence derived from a study involving 60 patients. Among this cohort, 87% exhibited a favorable therapeutic response after a solitary infusion. Within this responsive group, a substantial 62% achieved a comprehensive response characterized by the absence of any detectable neoplastic activity[15].

In the subsequent year, the European Medicines Agency (EMA) approved the immunotherapeutic agent named Abemca. This therapeutic modality is intended for employment in the adult population afflicted with multiple myeloma, a malignancy that has withstood a minimum of four distinct therapeutic regimens. These regimens encompass requisite components like an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody, each of which must have demonstrated either clinical efficacy or cessation of activity. The manufacturing protocol for Abemca parallels that of Yescarta and Kymriah, as does the array of associated side effects, albeit encompassing supplementary potential adverse reactions.A salient adverse effect attributed to Abemca pertains to hematological parameters, manifesting as a reduction in the count of various blood cell lineages, including erythrocytes, leukocytes, and platelets. This reduction precipitates sensations of debility and fatigue while concurrently elevating the propensity for hemorrhagic events, thereby amplifying susceptibility to infections. Consequently, diligent monitoring of blood counts ensues after treatment. Furthermore, the presence of Abemca within the bloodstream could potentially yield false-positive outcomes in certain routine clinical tests, including diagnostics for conditions such as HIV[16].

The clinical investigation that served as the foundation for FDA and EMA endorsement comprised a participant cohort numbering 100. Within this cohort, a substantial 72% exhibited a comprehensive response characterized by a meaningful attenuation in the indicators of myeloma. Additionally, 29% attained a complete response or surpassing it, thereby signifying the complete reduction of cancerous manifestations within the organism[16].

The latest endorsement by the Food and Drug Administration (FDA) pertains to Carvykti, an innovative form of immunotherapy involving Chimeric Antigen Receptor T (CAR-T) cells that target B cell maturation antigen (BCMA). This therapeutic intervention has garnered FDA approval for administration in adult patients grappling with recurrent myeloma that has manifested post-discontinuation of prior therapeutic interventions or has remained refractory. Carvykti's mode of action involves the directed recognition and engagement of BCMA, a protein ubiquitously present in myeloma cells.This interaction subsequently culminates in the selective eradication of BCMA-expressing cells, thus affecting the elimination of cancerous entities. This therapeutic strategy entails the genetic modification of the patient's T cells, endowing them to identify and assail BCMA situated on the surface of myeloma cells prevalent within the patient's biological milieu. Concomitantly, the manufacturing process mirrors Abemca's, paralleling the associated spectrum of side effects[17].

In the context of the clinical investigation, 97 participants volunteered for inclusion. Among this cohort, a noteworthy 78% attained a comprehensive response, denoting a remarkable reduction in myeloma indicators. Furthermore, the study unveiled a 77% progression-free survival rate within one year, accompanied by an overarching survival rate of 89%[17].

Overview of ongoing clinical trials and their results

In this clinical trial, researchers focused on investigating the role of the ORF57 gene in Kaposi's sarcoma-associated herpesvirus (KSHV) behavior. KSHV-infected cell lines, particularly those derived from primary effusion lymphoma, were employed as models to delve into the effects of disrupting the ORF57 gene. The study leveraged the revolutionary CRISPR/Cas9 technology, facilitated by a single vector carrying both the Cas9 enzyme and two guide RNAs. Through rigorous rounds of selection and isolation of single-cell clones, the scientists successfully deactivated the ORF57 gene in one of the clones.The impact of this intervention was twofold[18].

Firstly, the disruption of the ORF57 gene triggered instability within the KSHV genome, resulting in a marked reduction in viral genome copies and diminished expression of lytic genes. This observation sheds light on the critical role of the ORF57 gene in maintaining viral genome stability and optimal lytic gene expression. Secondly, the researchers extended their approach to cells with fewer KSHV genome copies, broadening the applicability of their method. This innovative technique not only showcases the feasibility of simultaneous Cas9 and dual guide RNA expression within a cell but also provides a versatile platform for precise genetic modifications across diverse genomes. This study marks the pioneering evidence of CRISPR's viability in silencing the ORF57 gene within the complete set of 100 KSHV genomes present in primary effusion lymphoma (PEL) cells. This accomplishment was realized by utilizing a co-expression vector with single-cell cloning[18].

In this clinical trial, the focus centers on overcoming drug resistance and inhibiting metastasis in breast cancer, critical challenges in cancer treatment. MicroRNAs (miRNAs) emerge as key players in regulating tumor development, but their potential to enhance therapy response and suppress metastasis remains underexplored. The study introduces miR-644a as a novel agent with multifaceted capabilities in breast cancer therapy. MiR-644a effectively hampers cell survival and epithelial-mesenchymal transition (EMT), a process linked to metastasis. Through intricate investigations, it becomes evident that miR-644a's presence and genetic attributes correlate with tumor progression and reduced risk of distant metastasis[18].

Mechanistically, miR-644a achieves its effects by targeting C-terminal binding Protein 1 (CTBP1), a co-repressor molecule. The CRISPR-Cas9 system was employed to knock out CTBP1, mirroring the impact of miR-644a. Consequently, tumor growth is suppressed, metastasis is curtailed, and drug resistance is diminished. Notably, miR-644a-mediated reduction of CTBP1 levels results in elevated functional wild-type or mutant-p53 proteins. These proteins act as molecular switches that steer the balance between G1 cell cycle arrest and apoptosis, a programmed cell death mechanism. The study establishes that the increase in mutant-p53, brought about by heightened miR-644a or reduced CTBP1, tips the balance towards apoptosis[18].

The clinical trials discussed here primarily focus on the application of CAR T-cell therapy in the context of glioblastoma (GBM) and triple-negative breast cancer (TNBC). In the GBM studies, CAR T-cell therapy's efficacy is explored, with a particular emphasis on identifying stable tumor-associated antigens (TAA) and optimizing T-cell subsets. Strategies such as co-expression of IL-8 receptors for enhanced T-cell trafficking and genetic engineering of CAR T-cells for bispecific targeting are investigated. These approaches demonstrate promising results in mouse models and advanced clinical trials, addressing challenges like tumor heterogeneity and off-target effects[18].

In the context of TNBC, CAR T-cell therapy exhibits potent targeting of tumor cells expressing specific antigens, such as tMUC1 and HER2. Modified CAR T-cells are designed to enhance immune response-related molecule production and suppress tumor cell proliferation. Notably, approaches involving NKG2D CAR T-cell co-stimulation and targeting HER family receptors show improved anticancer performance and offer potential avenues for overcoming cancer resistance. Specialized CAR T-cells targeting the biomarker mesothelin also hold promise in TNBC immunotherapy[18].

A trial focused on children and young adults with relapsed or refractory B-cell acute lymphoblastic leukemia (B-ALL) and utilized anti-CD19 CAR T-cells containing CD28 and TCR zeta domains. The therapy showed efficacy with manageable toxicities after the same chemotherapy regimen.Promising antitumor responses were observed using anti-CD19 CAR T-cells with a 4-1BB costimulatory domain in patients with non-Hodgkin lymphoma (NHL) or B-ALL. The inclusion of fludarabine conditioning chemotherapy improved overall response rates. Clinical trials combining anti-CD19 CAR T-cells with cyclophosphamide conditioning demonstrated enhanced clinical responses in patients with ALL and chronic lymphocytic leukemia (CLL). Relapses occurred due to low CAR T-cell persistence and the emergence of CD19-negative cells as an immune escape mechanism[18].

Interestingly, reports highlighted the efficacy of anti-CD19 FMC63-28Z CAR T-cells alone in treating various lymphomas and leukemias without prior chemotherapy, though graft-versus-host disease (GVHD) was observed in one patient. CAR T-cell therapies also showed promise as adjuvant treatments following autologous or allogeneic hematopoietic cell transplantation (HCT) in patients with ALL or B-cell NHL, with better outcomes seen with autologous HCT. Phase I and II trials of anti-CD19 CAR T-cells, specifically axicabtagene ciloleucel, demonstrated substantial anticancer responses in refractory NHL when combined with cyclophosphamide and fludarabine chemotherapy. Similar impressive results were seen with anti-CD19 CAR T-cells containing a 4-1BB costimulatory domain combined with chemotherapy in lymphoma patients[18].

Furthermore, CD20-specific second-generation CAR T-cells showed efficacy in driving refractory DLBCL into partial or complete remission when administered with prior conditioning chemotherapy. This underscores the potential of CAR T-cell therapy across various lymphomas and leukemias[18].

Challenges and setbacks faced in clinical trials

Some of the challenges faced in clinical trials with CAR-T cells include:

In Vivo Persistence and Functionality- Enhancing the long-term persistence and functionality of CAR T-cells within the patient's body is a significant challenge. Factors influencing this include conditions of T cell expansion in the laboratory, stability of transgene expression, and potential immune responses against the transgene. Prolonged persistence is crucial to prevent disease relapse. Then there is Therapeutic Toxicity; mitigating severe toxicities associated with CAR T-cell therapy is challenging. Factors contributing to these toxicities include the disease burden, high-dose chemotherapy regimen, infusion of high numbers of CAR T-cells, and elevated levels of serum cytokines and C-reactive protein. Addressing these toxicities is important for patient safety and treatment efficacy.Several critical aspects of CAR T-cell therapy remain unknown, such as the mechanism by which target cells are killed (possibly involving antigens or TCR complex chains), the fate of residual natural TCR, and the specific ways T cells mediate target-cell death[19].

Understanding these mechanisms is crucial for optimizing treatment strategies. Lastly is the optimal dosing and duration; determining the ideal dosage of CAR T-cells for individual patients is challenging, as responses vary. Some patients respond well to lower doses, while others require higher doses. Disease burden and toxicity levels also influence the response. Additionally, the duration of ex vivo T-cell expansion before infusion remains unclear, as prolonged expansion might not yield the best results. The efficacy of single vs. multiple infusions of CAR T-cells also requires further investigation[19].

There are also limitations to the use of CRISPR including:

CRISPR has off-target effects. As such one of the major limitations is the potential for off-target effects, where the CRISPR/Cas9 system can introduce unintended mutations in non-targeted regions of the genome. These off-target effects can result in unwanted changes in the function of genes, particularly concerning when targeting oncogenes and tumor suppressor genes. Despite efforts to modify gRNA length and structure, as well as the use of alternative strategies like nicking enzymes, addressing off-target effects without compromising efficiency remains challenging. Another is specificity and efficiency, the system's specificity is constrained by the requirement for a specific Protospacer Adjacent Motif (PAM) sequence and the high specificity of the target base. The catalytic window for editing is limited to around 4-5 nucleotides, which can lead to low efficiency and reduced precision in some cases. And lastly is its mosaicism and delivery challenges. Mosaicism can occur when cells divide during genome editing; mosaicism can occur, meaning that daughter cells might not carry the edited modifications accurately or consistently. Additionally, delivering the Cas9 protein as a ribonucleoprotein (RNP) in both in vitro and in vivo settings presents a significant challenge due to delivery efficiency and stability concerns[11].

Ethical and Regulatory Considerations

One of the implications of gene editing is that CRISPR-based RNA-targeted gene editing presents ethical concerns centered on potential nontarget effects. The persistence of gene drift within populations implies the ongoing propagation of off-target mutations across generations. Additionally, the augmentation of mutations in both quantity and impact as generations progress raises a broader ecological dilemma. Further ethical challenges emanate from the prospect of gene transfer to other species within ecosystems, potentially disseminating detrimental traits to associated organisms. The intricate distribution of gene properties among populations compounds difficulties in achieving effective control and mitigation[20].

Another is the application of CRISPR-Cas9 for human germline editing engenders multifaceted ethical inquiries. While its somatic cell application burgeons for enhancing traits, human germline editing remains prohibited due to safety concerns. This technique's capacity to modulate attributes such as athletic performance or behavior, rooted in genetic components independent of the environment, prompts ethical considerations. The quandary of procuring informed consent for minors, especially when intervening during zygote development, confers decision-making authority to parents or guardians for non-health-related reasons. Ethical discourse also revolves around the societal and moral dimensions of genome enhancement and its implications for societal inequalities[20].

Moreover, CRISPR technology's potential military use introduces ethical quandaries primarily within the realm of nontherapeutic enhancement. These ethical dilemmas are commonly scrutinized through the lens of risk-benefit analysis, informed consent, and accessibility. The pivotal concern lies in off-target mutations, which may lead to unintended genomic changes or deleterious consequences. The dearth of comprehensive information regarding off-target mutations necessitates meticulous evaluation of the benefit-risk balance. Moreover, apprehensions regarding CRISPR technology's potential dual-use for developing biological weapons raises ethical alarms within the military context.

Lastly, CRISPR technology introduces a compelling dimension of global inequalities, potentially exacerbating divisions among nations. The accessibility of CRISPR, coupled with its substantial costs, creates a scenario where developed countries could exploit the technology to fortify their defenses and possibly even engage in attacks against underdeveloped or developing nations. This disparity-driven dynamic threatens global peace and stability. It underscores the need for global governance mechanisms to navigate the ethical intricacies and implications of CRISPR technology, reflecting a pivotal intersection of science, ethics, and geopolitics[20].

The ethical framework for human genome editing is built upon key principles. These include prioritizing individual well-being through beneficial and safe applications, ensuring transparency and public involvement in policy-making, proceeding with due care and robust evidence, upholding responsible scientific practices, respecting individuals' autonomy and dignity irrespective of genetic attributes, promoting fairness in research benefits and risks, and fostering international collaboration while considering diverse cultural contexts. These principles collectively guide the responsible advancement of genome editing research and applications[21].

For example in the US, the Food and Drug Administration (FDA) oversees the regulation of gene therapies and other genetic interventions, including those using CRISPR technology. The FDA's regulatory framework involves evaluating these therapies through the Investigational New

Drug (IND) application process before they can be tested in clinical trials. The safety and efficacy of such interventions are rigorously assessed before approval for broader use[21].

In the EU, the European Medicines Agency (EMA) provides regulatory oversight for advanced therapy medicinal products, including gene therapies and gene-editing techniques like CRISPR. Developers are required to obtain a marketing authorization from the EMA before these therapies can be marketed and used within the EU member states. Ethical and safety considerations play a significant role in the evaluation process[21].

Or in China has been at the forefront of CRISPR-based human genome editing research. The country's regulatory framework has been under development to address the ethical and safety concerns surrounding gene editing. After the controversy surrounding the use of CRISPR-Cas9 to edit human embryos, China introduced guidelines requiring rigorous oversight and ethical approval for any human germline editing research[21].

Benefits and risks in the implementation of CRISPR-based therapies

Balancing benefits and risks is paramount in the implementation of CRISPR-based therapies, as these revolutionary tools hold immense potential for treating genetic disorders but also introduce various ethical and safety concerns. CRISPR-Cas9 allows for targeted modifications to the genome, offering a promising avenue for correcting genetic mutations underlying diseases. The benefits encompass the potential to cure or alleviate otherwise incurable genetic disorders, enhancing patients' quality of life and reducing the burden on healthcare systems. However, the pursuit of these benefits must be accompanied by a rigorous evaluation of associated risks. Off-target effects, unintended genetic changes, and immune responses pose potential dangers that could exacerbate the patient's condition or create unforeseen health issues. Thus, thorough preclinical studies, robust monitoring mechanisms, and long-term follow-up are essential to mitigate these risks and ensure that the potential benefits outweigh the potential harms[22].

Effective balancing of benefits and risks requires a comprehensive risk assessment process that acknowledges the uncertainty inherent in cutting-edge technologies like CRISPR. Early-stage clinical trials should emphasize safety, selecting target conditions where the risk of adverse effects is minimal and the likelihood of therapeutic success is relatively high. As therapies advance to more complex conditions, a careful consideration of the risk-benefit profile becomes imperative. Informed consent processes should provide patients and their families with a clear understanding of both the potential benefits and uncertainties surrounding CRISPR-based interventions. Moreover, a transparent and collaborative approach between scientists, clinicians, ethicists, and regulatory bodies is essential to ensure that decisions are made with a balanced perspective, incorporating diverse viewpoints and expert opinions[22].

Future Prospects and Challenges

CRISPR technology's precision gene-editing capabilities offer a groundbreaking avenue for cancer therapy. Researchers are working on targeting specific cancer-associated genes with the goal of disrupting their function or introducing therapeutic modifications. By focusing on oncogenes that drive cancer growth or tumor suppressor genes that regulate cell division, CRISPR could potentially halt or slow down cancer progression. This approach not only provides a means to directly tackle the root causes of cancer but also offers the potential for highly personalized treatment strategies tailored to the genetic makeup of individual patients[4].

CRISPR's impact on cancer therapy extends to immunotherapy, where it has the potential to significantly enhance the effectiveness of treatments like CAR-T cell therapy. Scientists are utilizing CRISPR to engineer patients' immune cells to better recognize and attack cancer cells. By precisely modifying immune cells to express chimeric antigen receptors (CARs) that target specific tumor antigens, researchers can create CAR-T cells that are more potent and durable in their anti-cancer response. This advancement could lead to improved outcomes and broader applicability of immunotherapies, benefiting patients with various types of cancer[22].

CRISPR technology opens the door to exploiting synthetic lethality in cancer cells, a phenomenon where the simultaneous disruption of two genes leads to cell death. By systematically identifying gene pairs that exhibit synthetic lethality in the context of cancer-associated mutations, researchers can design targeted interventions. This approach could pave the way for innovative therapies that selectively eliminate cancer cells while sparing healthy tissue. Harnessing synthetic lethality using CRISPR-based strategies holds great potential for enhancing the precision and effectiveness of cancer treatment[22].

CRISPR's versatility extends to personalized cancer vaccines and early detection methods. Researchers are exploring the use of CRISPR to modify cancer cells, enabling them to express tumor-specific antigens on their surfaces. These modified cells could be used to create personalized cancer vaccines that stimulate the immune system to recognize and attack the cancer. Additionally, CRISPR-based diagnostic tools provide highly sensitive methods for detecting cancer-associated mutations in DNA or RNA. This breakthrough could revolutionize cancer detection, allowing for earlier diagnosis and more accurate monitoring of treatment responses, ultimately improving patient outcomes[22].

Addressing the challenges and limitations for broader adoption

As it has already been mentioned one of the major pitfalls of the CRISPR is it’s off-target, which can work opposite and make cancerous cells as it happened in 2002 study/clinical trial. One way that they can try to reduce this major pitfall includes increasing the specificity of nucleases, such as Cas9, which are responsible for DNA cleavage. This can be achieved by engineering the Cas proteins to improve their targeting accuracy. Different Cas proteins that exhibit enhancements in on-target specificity have been engineered, including eSpCas9, HF-Cas9, HypaCas9, and Sniper Cas9. These engineered Cas proteins have been designed to minimize off-target cleavages while still maintaining effective on-target activity. Another strategy is to use Cas9 nickases, where one of the endonuclease domains of the Cas9 protein is inactivated. This results in a reduced ability to cause double-strand breaks in the genome, leading to lower off-target effects. By introducing only single-strand breaks, the risk of incorrect repair and off-target mutations is diminished. This approach has been shown to significantly decrease off-target effects in genome editing experiments[23].

Another main issue of gene- editing approaches is the requirement for a specific PAM (Protospacer Adjacent Motif) sequence adjacent to the target site. The availability of suitable PAM sequences can limit the choice of target sites. However, the advancement of Cas-nucleases such as SpCas9 and Cas12a, with varying PAM requirements, has expanded the range of targetable loci. This increased PAM flexibility enables researchers to edit genes at more specific target sites, providing greater flexibility in genome editing[23].

The future impact of CRISPR in cancer treatment is poised to be revolutionary, reshaping the landscape of how we understand and combat the disease. CRISPR's unparalleled precision in gene editing holds the potential to unlock novel therapeutic strategies. Researchers envision a scenario where cancer cells are reprogrammed using CRISPR to revert to a more benign state or undergo cell death. By targeting specific genetic alterations driving malignancy, CRISPR could essentially 'edit out' the cancerous traits, leading to innovative curative approaches. This approach could be particularly promising for aggressive or metastatic cancers that have proven resistant to traditional treatments[24].

A significant aspect of CRISPR's future impact on cancer treatment lies in its potential to facilitate truly personalized therapies. Each patient's cancer is unique due to its genetic makeup, and CRISPR's ability to precisely target individual genetic aberrations could result in tailored treatments tailored to the patient's molecular profile. This might involve editing cancer cells to sensitize them to existing therapies, enabling more effective treatment responses. Furthermore, CRISPR's potential in developing targeted therapies could minimize off-target effects and reduce the collateral damage to healthy tissues often associated with conventional treatments, leading to fewer side effects and improved quality of life for patients[24].

Drug resistance and cancer relapse are persistent challenges in oncology. CRISPR's impact on cancer treatment could extend to overcoming these hurdles. Researchers foresee a future where CRISPR is used to edit cancer cells, rendering them susceptible to previously ineffective treatments. By targeting the mechanisms that drive resistance, such as specific mutations or altered gene expressions, CRISPR could potentially extend the efficacy of existing therapies. Moreover, CRISPR might play a crucial role in preventing relapse by eliminating residual cancer cells that can evade current treatments. This approach could lead to more durable remissions and increased long-term survival rates for cancer patients[24].

Conclusion

In this comprehensive review, we provide a concise overview of the CRISPR/Cas9 system, covering its structural and functional aspects in different phases. We then focus on its application in cancer immunotherapy, particularly with regard to TCR, TIL, and CAR-T cells, highlighting the engineering of CAR-T cells for precision cancer targeting, exemplified by FDA-approved therapies like Yescarta and Kymriah. Ethical considerations in CRISPR/Cas9's use in cancer therapy are discussed, along with its diverse applications in degenerative diseases, viral infections, genetic disorders, pathogen detection, and agriculture. Challenges related to in vivo delivery for precise cancer gene targeting are also addressed.

The advent of CRISPR/Cas9 technology has emerged as a pivotal turning point in the landscape of cancer treatment, underscoring its profound potential to revolutionize therapeutic paradigms. By virtue of its precision and adaptability, CRISPR/Cas9 offers the prospect of targeting malignancies at the genetic level with an unprecedented level of accuracy, thereby mitigating off-target effects and enhancing therapeutic efficacy. Furthermore, CRISPR-based approaches hold the promise of tailoring treatments to the genetic profiles of individual patients, fostering a new era of personalized oncology interventions. As the field of oncological research continues to harness the capabilities of CRISPR, its transformative influence on cancer treatment strategies is poised to be increasingly profound and far-reaching.

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